THESIS

Cellular and molecular mechanisms involved in the morphological and functional alterations of the renal glomerulus in aging and hypertension

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KEYWORDS:
Renal cortex; Renal glomerulus; Podocytes; Mesangial cells; Physiological aging; Hypertension; Cellular senescence; Senescent phenotype; Cell cycle; Oxidative stress; Reactive Oxygen Species (ROS); Mitochondrial dysfunction; Endoplasmic reticulum stress; Autophagy; Senescence associated lysozomal β-galactosidase (SA-β-gal), L-NAME ("N°-Nitro-L-arginine methyl ester hydrochloride"); Zofenopril; Antioxidant
ABSTRACT

The thesis entitled "Cellular and molecular mechanisms involved in the morphological and functional alterations of the renal glomerulus in aging and hypertension" is aimed to identify new molecular mechanisms associated with the development of cellular senescence of the renal glomerular cells in physiological aging and hypertension. The content of this thesis is structured in two parts: "The current knowledge" and "The original Contributions".

Worldwide the proportion of people over 60 years is growing faster than any other group. According to the World Health Organization (2012), by 2050 the population of people over 60 will double, as compared with the year 2000. Aging is not a disease, but advancing age is associated with increased vulnerability to chronic diseases, such as hypertension, atherosclerosis, heart failure. The health care needs and comorbid health problems of the elderly require a special focus, and impose a substantial pressure on publicly-funded healthcare system. The subject of the thesis research is important because the understanding of the mechanisms of aging will contribute to developing novel strategies for increasing the quality of life of the elderly, in order to maximize their health and functional capacity, as well as their social participation.

In physiological aging, morphological and functional changes of the kidney contribute to the development of chronic kidney disease. The accumulation of senescent cells has been implicated in driving aging-related tissue alterations. Thus, targeting of senescent cells during the course of normal aging is expected to improve the tissue milieu, thereby preventing the malfunction of the remaining nonsenescent cells. Recently, using a progeroid mouse model it has been shown that p16Ink4a-positive senescent cells drive age-related pathologies, and that selective removal of these cells can delay tissue dysfunction and the aging phenotype (Baker et al., 2011). Studies on renal cell senescence could lead to development of new therapeutic approaches for delaying age-associated dysfunctions of the aging kidneys, extending the health span.

The 1st part of the thesis is consisted of three chapters. Chapter I contains general concepts on the kidney anatomy and physiology, with special emphasis on the renal glomerular anatomy and physiology, covering the architecture of the glomerular filtration barrier, and the mechanism of glomerular ultrafiltration. Chapter II refers to the pathological changes of glomerular cells and related glomerular dysfunction. The alterations of the glomerular endothelial cells, such as alterations of their fenestrated phenotype, changes of the glycocalyx, or cellular alterations due to the blood flow stress
are detrimental for the glomerular filtration. Also, the dysfunctional glomerular filtration barrier is caused by: i) structural changes in the glomerular basement membrane, ii) structural and functional alterations of podocytes (such as the effacement of their pedicels, or the hypertrophy, or even the cells loss), and iii) morpho-functional alterations in the mesangial region (the proliferation, or apoptosis of the mesangial cells, and mesangial hyperplasia). In Chapter II are highlighted the morphological and functional alterations of the renal glomeruli in aging and hypertension. Assuming that one of the leading cause of changes in the glomerular basement membrane architecture, and of the podocytes’ alterations in the aging kidney is represented by the accumulation of the senescent cells, in Chapter III are revealed those proteins involved in intracellular signaling responsible for the senescent phenotype: the markers and effectors of cellular senescence. In this chapter is highlighted the relevance of cellular senescence in the physiological aging process. In its "early" stage, the cellular senescence is a protective mechanism against oncogenic conversion of the cells, but exacerbation of the cell senescence-associated secretory phenotype has negative impact on the neighboring non-senescent cells.

The second part, named Original contributions, comprises the original results obtained in three studies focused on: 1) identifying senescent cells in renal glomeruli from 24 months old Golden Syrian hamsters (aged hamsters), or from hamsters with L-NAME-induced hypertension; 2) deciphering the cellular and molecular mechanisms involved in the setting of the cellular senescence in renal glomeruli, and 3) the improvement of the oxidative stress induced alterations in renal cortex, by Zofenopril administration to L-NAME-induced hypertension rats.

In the first study, named "Cellular and molecular mechanisms involved in cell senescence in the kidney glomerulus in aging", we have investigated the molecular mechanisms involved in the onset of cellular senescence in physiological aging, targeting the development of new therapeutic strategies to delay the occurrence of chronic renal disease for the improvement of the quality of life of older adults. We used as experiment model the Golden Syrian hamsters from which we harvested the kidneys. The experiments were performed on 24 months old (considered aged hamsters), and 4 months old (young) hamsters, respectively, and the renal cortex was processed for: i) light microscopy (on frozen and paraffin sections), and electron microscopy, for identifying glomerular cells alterations in aging, mitochondrial dysfunctions, endoplasmic reticulum stress, and the autophagy; ii) the activity of the lysosomal
β-galactosidase, known as senescence-associated β-galactosidase (SA- β-gal), at pH 6; iii) the immunochemical labeling of the proteins of interest, either by Western blotting, or by immunohistochemistry (fluorescence microscopy); iv) estimation of the reactive oxygen species production, related to the oxidative modification of proteins, and the stress-activated signaling pathways involved in the regulation of the cell senescence.

Histological examination of the kidney sections stained with hematoxylin-eosin, PAS, Masson’s trichrome, or methenamine silver, revealed the presence of the focal and segmental glomerulosclerosis, the excessive accumulation of type IV collagen and the thickening of the basement membrane in the glomeruli from aged hamsters. The activity of SA β-gal at pH 6 revealed the presence of senescent cells in renal glomeruli from aged hamsters (Fig. 1, left). So far, there are only few data reporting the presence of senescent cells in the kidney. Senescent cells are irreversible growth-arrested in the G1 phase of the cell cycle, and p16\text{\textsuperscript{INK4a}}, a cyclin-dependent kinase inhibitor, enforces growth arrest, being recognized as a biomarker for senescence. The increased expression of p16\text{\textsuperscript{INK4a}} in the renal cortex from aged hamsters was evidenced by Western blotting, and by immunofluorescence microscopy (Fig. 1, right).

Senescent cells usually adopt a flattened appearance in vitro, and, while not dividing, remain metabolically active, displaying characteristic changes in gene expression. These cells are characterized by a pro-inflammatory phenotype, possibly leading to detrimental effects on tissue homeostasis (Passos et al., 2010). The increased expression of p27\text{\textsuperscript{Kip1}} and p21\text{\textsuperscript{Cip1}} (the Cip / Kip family of cell cycle inhibitors which get in the way of the cell cycle G1 phase complexes arresting the cell cycle before S phase) has revealed the permanent growth arrest of the renal cells from aged hamsters in the G1 phase of the cell cycle, figuring the senescence of these renal cells. Also, the Western blot studies indicate the tissue accumulation of CDK4 in the renal cortex of the aged hamsters.
Senescence is a cellular response to various stress factors that induce the DNA damages, and post-translational modifications of the protein, governing the activation of the two main signaling pathways mediated by ARF/p53/p21Cip1 and p16INK4a/pRb. Determination of the reactive oxygen species (ROS) production in the renal cortex homogenates from young and old hamsters showed increased generation of ROS in old hamsters that could be in charge for the launching of cellular senescence in renal glomeruli. The rising of the oxidative stress in the kidney of the aged hamsters may be the cause of the increased protein expression of p53, a redox-sensitive transcription factor involved in the cell decision to become senescent.

The alarmin HMGB1 ("high mobility group box 1") is another protein recently identified as a mediator of senescent phenotype due to the role it plays in the chromatin remodeling and for its property to enable the transcription factors to bind the nuclear DNA, (Davalos et al., 2013). In early phases of senescence, HMGB1 translocate from the nucleus to the cytosol, subsequently being secreted outside the cell, both translocation of HMGB1 from the nucleus, and its redistribution into the extracellular space depends on p53 activation. Taking into account the involvement of HMGB1 in senescence, we showed the reduction of the HMGB1 protein expression in the renal cortex from the aged hamsters (Fig. 2, left, Western blot) and the translocation of HMGB1 from the nucleus to the cytosol of the glomerular cells from aged hamsters (Fig. 2, right, immunofluorescence).

There is a causative link between oxidative stress, mitochondrial dysfunction, cellular senescence and aging phenotype (Velarde et al., 2012). In our study, the electron microscopic examination of the glomerular cells from aged hamsters revealed dysfunctional mitochondria, both in podocytes (Fig. 3, left) and mesangial cells (Fig. 3, right).
The mitochondrial dysfunction is the cause and the consequence of the cellular senescence due to the increased oxidative stress and the failure of antioxidant enzymes to remove ROS. The estimation of the protein expression of mitochondrial MnSOD (a mitochondrial enzyme playing defensive role against ROS) in renal cortex homogenates from aged hamsters versus young ones does not indicate significant changes in the expression of this enzyme, but there is the possibility to encounter the decreased enzymatic activity of MnSOD from aged hamsters, so that the enzyme might be ineffective in reducing oxidative stress.

The electron microscopic examination of the glomerular podocytes and mesangial cells from aged hamsters have revealed the presence of lipofuscin, a non-degradable product resulted through the crosslinking / aggregation of proteins and peroxidized lipids. Lipofuscin accumulation occurs specifically in aging. Dysfunctional mitochondria, which release highly reactive hydroxyl anion, may contribute to the establishment of lipofuscin. The high number of damaged mitochondria suggests a decrease of autophagy / mitophagy (which normally remove damaged mitochondria) in the glomerular podocytes and mesangial cells from aged hamsters.

Mitochondrial function is adjusted by a series of protein. AMP Kinase, the energy metabolism sensor, triggers the activation of mitochondrial biogenesis, and targets the dysfunctional mitochondria for degradation through the mitophagy (Hardie, 2011). The mitochondrial dysfunction and the increased production of ROS contributes to the enhancement of the AMP content of the cells. The rising of the ratio AMP: ATP in the cells promotes the activation of the AMP Kinase that regulates metabolic processes in
order to save the cellular energy. The AMP kinase play a role in the initiation of senescence as it is suggested by the ability of the AMP kinase to activate the p53 tumor suppressor. The protein expression of AMP Kinase was increased in the renal cortex from aged hamsters by comparison with the young, suggesting the involvement of this kinase in the aging process and in the onset of cellular senescence in the glomerulus.

Two other proteins were investigated for their involvement in cellular senescence and tissue aging: SIRT1 and Nampt. The activation of SIRT1 and Nampt, together with an optimal intracellular concentration of NAD+, play an important role to ensure the longevity of organisms. SIRT1 is an enzyme that deacetylates proteins using NAD+ as substrate, and it plays a key role in cellular metabolism and in regulation of many cellular processes, from cell survival to cell response to various stress factors. Western blot analysis of the renal cortex homogenates from young and aged hamsters revealed the two times decrease of the protein expression of SIRT1 in aged hamsters versus young, a result that correlates with the increasing of the oxidative stress and with the alteration of the mitochondrial structure. Nampt is an enzyme responsible for the cellular synthesis of NAD+, which contributes to the recovery of NAD+ bioavailability, with beneficial effects on cellular NAD+ and ATP content. Determination of Nampt expression in the renal cortex of aged and young hamsters showed the significant decline of Nampt expression in aged hamsters related to the young ones.

Finally, the results of the experiments that we conducted on kidneys from aged and young hamsters showed that:

- in the aging process, the initially stage of glomerulosclerosis seems to be regarded as glomerular cell hypertrophy, which is followed by the thickening of the glomerular basement membrane, by the mesangial matrix expansion, the moderate atrophy and glomerular capillary tuft;

- the glomerular cells from aged hamsters express markers of cellular senescence: SA-β galactosidase activity at pH 6, increased expression of p16\textsuperscript{INK4a} and permanent arrest of the cell in G1 phase of the cell cycle, both of them are induced by increased expression of p27\textsuperscript{Kip1} and by the accumulation of CDK4 inside the cell;

- the increased levels of reactive oxygen species in the renal cortex from the aged hamsters could be involved in the activation of the intracellular signaling pathway mediated by p53/p21\textsuperscript{Cip1};

- the alarmin HMGB1 contributes to the initiation and stabilization of the senescent phenotype;
the accumulation of dysfunctional mitochondria in renal glomerular cells from aged hamsters could contribute to lipofuscin development, and to the setting up of the senescent phenotype;

the increase of the AMP Kinase expression, maybe due to the augmented oxidative stress, is indicating an energetic stress associated with aging, this stress causes the reduction of the SIRT1 and Nampt expression in the renal cortex from aged hamsters.

The second study included in the thesis, called "Molecular mechanisms of cellular senescence related to L-NAME-induced hypertension" aimed: i) to shed light on the presence of senescent cells from the renal glomeruli in hypertension, a disease depicted by increased oxidative stress and ii) to reveal the intracellular mechanisms involved in the in vivo settings of the senescent phenotype in hypertension. High blood pressure (hypertension), known as "the silent killer", is a major public health problem affecting approximately one billion people worldwide. The presence of the senescent cells in glomerular diseases associated with hypertension is not yet assessed, but it is assumed that the decreased bioavailability of nitric oxide (NO) is involved in the pathogenesis of hypertension and contributes to increased oxidative stress and to the occurrence of senescent phenotype. Chronic inhibition of NO synthase by L-arginine analogues such as L-NAME induces senescence of endothelial cells in vitro while in vivo and this lead to hypertension and to arteriolosclerosis (Zhong et al., 2010). The role of NO in cellular senescence is still unclear, and the reports from the literature on this subject are scarce.

The experimental design of this study included an original experimental model: the golden Syrian hamster with hypertension induced by chronic administration of L-NAME (an inhibitor of NO synthesis). The group of hypertensive hamsters (HT) consisted of male hamsters receiving L-NAME (45 mg / kg / day, 200 μl) by gavage for 35 weeks, while the control group (C) was made up of males the same age as those of the HT who received daily 200 μl serum physiological. The main purposes of the study were: i) the hemodynamic and biochemical characterization of the experimental model, ii) the histological evaluation of the changes induced by L-NAME in the renal glomeruli, iii) to emphasize, by electron microscopy, the changes of the ultrastructure of the renal glomeruli from the HT and C groups of hamsters, iv) the identification of the senescent cells based on the lysosomal β - galactosidase associated with senescence (SA-β-gal) with activity at pH6 which are present in the renal glomeruli from hypertensive hamsters, v) the assessment of the reactive oxygen species production in the renal
cortex harvested from hamsters with hypertension or from control, vi) the estimation of the oxidative modification of proteins in the glomerular cells from hamsters with hypertension or control, vii) to quantify of the protein expression of the key players molecules that regulate the stress- activated signaling pathways involved in induction of cellular senescence.

**The results** of this study showed that chronic inhibition of NO synthesis with structural analogue of L- arginine (L-NAME) induced hypertension (HT) to golden Syrian hamsters. The hypertension showed up early stages glomerulosclerosis, without fibrosis or hyaline tissue deposition, without glomerular basement membrane thickening, but accompanied by the collapsed glomerular capillaries and by the effacement of the pedicels. Determination of the *superoxide anions* production in the renal cortex homogenates from hypertensive hamsters versus control showed an increase by ~ 90 % of the superoxide anions synthesis in hypertensive hamsters. The oxidative stress contributes to oxidative alterations of proteins, lipids and cellular DNA. The protein carbonyl content is an indicator of protein oxidation in the settings of oxidative stress. The carbonylated proteins may be involved in the induction of cellular senescence, as was recently proven by the senescent fibroblasts (Baraibar et al., 2012). Spectrophotometric determination of the *protein carbonyls* in the renal cortex of hypertensive hamsters versus control showed an increasing in the oxidative modifications of proteins in the homogenates from hypertensive hamsters group. The augmented production of superoxide anion and the oxidative modification of proteins through carbonylation in the renal cortex from hamsters with hypertension may contribute to the launching of senescence in glomerular cells. In this study we identified senescent cells positive for β - galactosidase activity (SA- β - gal) at pH6 in the glomerular cells of the hypertensive hamsters.

The activation of p53 and of the protein substrate p21^{Cip1} contribute to the cell cycle arrest in G1 phase and to the induction of the cellular senescence. The protein expression of p53 redox-sensitive transcription factor was increased in the renal cortex from hypertensive hamsters (~ 150 %) related to the control. The elevated levels of protein expression of p53, p21^{Cip1} and p27^{Kip1} were founded in the renal cortex of hypertensive hamsters, suggesting the irreversible arrest of the cell cycle in G1 phase, one of the main features of the cellular senescence. Also, the activation of AKT protein kinase in the glomeruli from hypertensive hamsters can induces the accumulation of p53 and p21^{Cip1} in the cells, contributing to the launching and stabilization of cellular senescence in renal glomerular cells.
In the late, this study showed that cellular senescence is induced in glomerular cells in the setting of chronic inhibition of NO, perhaps due to the increased oxidative stress that activates intracellular signaling pathway mediated by AKT/p53/p21Cip1. Pharmacological targeting of the molecules involved in the settings of the glomerular cell senescence in the hypertension, could be the basis for the development of new therapeutic strategies to tackle hypertension.

The third study of the thesis, entitled "The involvement of the antioxidant effect of Zofenopril in restoring glomerular dysfunction induced by chronic inhibition of NO synthesis" was focused on the molecular mechanisms linked to antioxidant action of Zofenopril in the renal glomerulus in the experimental model of L-NAME induced hypertension in Wistar rats. The kidneys play a critical role to keep the regular blood pressure range, and high blood pressure leads to chronic kidney disease. Oxidative stress is a key mediator in worsening of the renal lesions associated with hypertension. The main cellular source of ROS is NADPH oxidase in the kidney, the enzyme consists of two membrane anchored subunits (gp91phox and p22phox) and four cytosolic subunits (p40phox, p47phox, p67phox and NOX, Williams et al., 2007). The ROS level initiate the activation of different redox - sensitive transcription factors. A significant amount of ROS triggers an inflammatory response through activation of NFkB and AP -1 (Reuter et al., 2010). Zofenopril, an inhibitor of angiotensin converting enzyme, which blocks the conversion of angiotensin I to angiotensin II and lowers blood pressure, is accomplished of chelating free radicals due to its free sulfhydryl group, giving anti-oxidant properties to this drug. There are reported only few data about the effects of Zofenopril in the background of the increased oxidative stress in the kidney. The experiments settled down for this study were accomplished using the male Wistar rats, 3 months old, randomly grouped into: i) HT group includes rats became hypertensive after the receiving of L -NAME (50 mg / kg / day) in drinking water for 12 weeks, ii) HTZ group of hypertensive rats that received L -NAME (50 mg / kg / day ) 8 weeks and for the next 4 weeks of treatment the rats received both the L-NAME and Zofenopril ( 15mg / kg / day), and iii) C, the control group consists of rats of the same age with the HT and HTZ groups, they did not received L-NAME or Zofenopril. Our study aimed to investigate: i) the glomerular alterations induced by chronic administration of L-NAME to the Wistar rats, ii) the superoxide anion production and NADPH oxidase subunits expression (as the main source of supeoxide anions) in the renal cortex of HT, HTZ and C rats, iii) the oxidative modifications of proteins by quantifying protein carbonyls levels, iv) the protein expression of NF-kB subunits which
are adjusted by reactive oxygen species, and v) the levels of reduced (GSH) and oxidized glutathione (GSSG) together with the glutathione peroxidase activity (GPx) to evaluate the efficiency of intracellular antioxidant system, and the ratio of GSH / GSSG which is a sensitive indicator of the redox state. Our results showed that:

- the administration of L-NAME to the Wistar rats (HT group) leads to:
  i) the increased blood pressure and angiotensin converting enzyme activity, ii) the ultrastructural changes of endothelial cells and podocytes in glomeruli of HT rats (including loss of the fenestrae, the seldom loss of filtration diaphragm between adjoining pedicels and the effacement of the pedicels); these changes disturbed the properties of the glomerular filtration barrier, iii) the increased production of superoxide in the renal cortex of HT rats, iv) the increase of the oxidative stress, as evidenced by increased levels of protein carbonyls (as a marker for protein oxidation) and by the activation of NF - kB (as intracellular redox - sensitive signaling pathway) in the renal cortex of HT rats, v) the diminishing in the antioxidant capacity of the renal cortex of HT rats assessed by the decrease in GSH content and by the decline of GPx activity.

- Zofenopril administration to the rats with hypertension induced by L-NAME had a beneficial effect on ultrastructural changes and induced the recovery of some components of oxidative stress: the concentration of superoxide anion, the protein expression of NADPH oxidase subunits (p22phox, Nox1, p47phox, p67phox), the protein expression of NFkB subunits p50 and p65, respectively, and the decreasing of the protein carbonylation.

Lately, Zofenopril administration to the rats with hypertension induced by L-NAME has renoprotective effects, contributing to the decrease of the local oxidative stress, to alleviate the changes caused by this oxidative stress and to restore the antioxidant capacity of the cells through the improving the glutathione content and glutathione peroxidase activity in the renal cortex.

GENERAL CONCLUSIONS

Physiological aging induces morphological and functional alterations of all organs, including the kidneys. In the last decade the aging phenotype was associated with the accumulation of senescent cells in tissues. Some of the components secreted by senescent cells induces alterations of surrounding tissue structure and function, thus contributing to tissue dysfunction associated with aging.

The results gained from our studies have shown the presence of senescent cells in the renal glomerulus from aged hamsters and from hamsters with hypertension.
induced by chronic inhibition of nitric oxide synthesis. The experiments performed on the renal cortex from aged hamsters and hypertensive hamsters suggests that the glomerular cells senescence is associated with mitochondrial dysfunction and contribute to alleviation, or dysfunction of autophagy, mainly in the podocytes. Our data substantiate that the oxidative stress and the irreversible arrest in G₁ phase of the cell cycle are two of the key steps in the onset of cellular senescence in the kidney glomerulus, both in aging and in hypertension. The molecular mechanisms that mediate these two processes are different and depend on their activation settings. The activation of redox - sensitive tumor suppressor p53 plays key role in mediating the signals inducing the cellular senescence surrounding of the increased oxidative stress. The cellular and molecular mechanisms involved in the launching of the cellular senescence in renal glomerulus in aging and hypertension are not completely deciphered, so the studies included in this work come to support the existing issues in the literature that address the same topic.

Bibliography:


