Review Article

Ursolic acid: a versatile triterpenoid compound in regulating the aging

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Abstract

We and other studies have elucidated single molecules that can attenuate aging and extend longevity. Indeed, these molecules could prevent age-associated diseases simultaneously and probably extending healthy-life spans. In this review, we discuss recent advances, controversies, opportunities and challenges surrounding ursolic acid (UA) in relationship with aging. In this regard, UA also known as urson, prunol, micromerol and malol is a pentacyclic triterpenoid compound which naturally occurs in a large number of vegetarian foods, medicinal herbs and other plants, including apples and rosemary. Our previous studies for the first time evaluated anti-aging effects of UA. These studies indicated that UA through overexpression of anti-aging biomarkers such as sirtuin (SIRT) 1, SIRT6 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha/beta (PGC-1α/β). Klotho and orexin-A in the brain, skeletal muscle, liver and kidney ameliorates aging and thereby conferring broad health benefits in rodents, primates and possibly human. Taken together, our recent findings advantageously described that UA mimics short-term caloric restriction, exercise and has a potential therapy for age-related diseases. Finally, the prospects for drugs that can deliver at least a subset of the benefits of caloric restriction seems very real.

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Introduction

Aging is a complex degenerative process characterized by a metabolic functional decline and increase risk of related disease, including type 2 diabetes mellitus, cardiovascular disease and stroke which ended to death (Ford et al., 2002). Many diseases processes and environmental factors profoundly influence the rate of aging. Furthermore, the aging process occurs at different rate among different tissues and the functional manifestations also vary (Nair, 2005). Aging-related changes in one organ might affect the functions of other organs (Nair, 2005). Only 20 years ago, the idea that a drug could treat one disease and prevent a dozen others was considered fanciful by many scientists. Today, abundant findings have shown that multiple diseases of aging can be prevented by small molecules in rodents (Nair, 2005) and primates (Fiori et al., 2013). Given their obvious role in mediating the health benefits of natural compounds, and the subsequent exhibition of therapeutic value in preclinical animal models, sirtuins (SIRTs) have attracted substantial interest as drug targets (Blum et al., 2011). Most studies declared that the widespread using would come from molecules that activate/up-regulation one or more of sirtuins, and inhibition of their also shows promise in some cancer models (Morris, 2013). One of the outstanding effects of these compounds is their

capability to avoid and reverse the effects of obesity and age-related metabolic disorders (Minor et al., 2011). But there is still a long avenue ahead to develop an approved drug in order to improve health and survival of mice on a sirtuin activating compounds. Both inhibitors and activators have been discovered for sirtuins; some act specifically, other across the entire family (Morris, 2013). Of all the natural SIRT1 activators discovered to date, ursolic acid (UA) is still the most potent *in vitro* and *in vivo.* UA is a natural triterpene, pentacyclic and lipophilic compound revealing many pharmaceutical properties (Liu, 1995). In this review we attempted to describe an overview of knowledge about the anti-aging and pharmacological properties of ursolic acid. For first time we evaluated UA effects on the expression of anti-aging biomarkers such as SIRT1, SIRT6, peroxisome proliferator-activated receptor gamma coactivator 1-alpha/beta (PGC-1α/β) and klotho in the brain, skeletal muscle, liver and kidney. Besides, in a recent survey we considered neuroprotective effects of UA through scrutinizing of orexin-A. Finally, this review does not attempt to be a comprehensive review of all findings in the area of ursolic acid, and we apologize to those whose work is not explained.

An overview of ursolic acid

Ursolic acid (3β-hydroxy-urs-12-ene-12-ene-28-oic, Fig. 1) is a phytochemical widely distributed in a variety of food products and herbs, most well known for being in apple peels (Frighetto et al., 2008). Scientific researchers evaluated the biological activities of UA. They evaluated the pharmacological properties of UA and reported the very poor bioavailability (~ 0.6 bioavailability) (Yang et al., 2012). Moreover, it appears UA is not a significant concern for cardiovascular health and may be also protective (Tannock, 2011; Ullevig et al., 2011); although more studies are needed to confirm this issue. It is reported that UA influences insulin receptor, possibly through the protein-tyrosine phosphatase 1B enzyme, and augments insulin effect on the receptor (Jung et al., 2007; Kunkel et al., 2012b). UA seems to be beneficial either by itself or in conjunction with anti-diabetic agents for reducing serum glucose over an experimental period (Sundaresan et al., 2012). In addition, low to moderate doses of UA also appear to protect rats from immune-system related side effects of diabetes

and may offer putative protective effects (Zhou et al., 2010). Supplementation of UA is thought to increase circulating levels irisin, a peptide secreted from a few organs (including skeletal muscle) that browns adipose tissue and may have antiobese effects. More research is needed to confirm this function of UA (Kunkel et al., 2012a; Huh et al., 2014). It has been shown that low dosages of UA emerge to have beneficial effects on skeletal muscle, but higher dosages have been implicated in preserving muscle mass during fasting (Figueiredo and Nader, 2012). To examine inflammatory and immunology effects of UA, it reduces cortisol, but this has not yet been tested in a living system (Rollinger et al., 2010). It is noted that UA decreases leptin, but these results may be influenced by weight loss (Jang et al., 2010). One study showed that utilizing of 150 mg of UA three times a day for eight weeks was able to increase circulating insulin-like growth factor 1 concentrations by 22.8% relative to placebo (Bang et al., 2014). In the deliberation of UA interaction with organ systems, it should be noted that UA through suppression of both testosterone and dihydrotestosterone rivalling Finasteride in magnitude leads to reduce serum levels of prostate specific antigen (Shin et al., 2012). Furthermore, in studies measured liver enzymes, there is no increase following 5 mg/kg oral ingestion for 4 weeks (Shin et al., 2012). According to angiogenesis inhibition effects of UA, it also inhibits expression of matrix metalloproteinase 2 and 9, intermediates required for the final stages of angiogenesis into new tissue (Kanjoormana and Kuttan, 2010). In the final scenario of biological effects of UA about safety and toxicity, *in vitro* studies demonstrated that UA was able to reduce sperm motility (Chattopadhyay et al., 2005). Based on these considerations, our recent studies for first time elucidated the anti-aging effects of UA in crucial tissue such as brain, skeletal muscle, liver and kidney.

UA effects in aging brain

The hypothalamus is a small structure, critical anatomical site, located in the ventral diencephalon in which cells senses changes in energy status of the body and coordinate responses aimed at maintaining metabolic homeostasis. Recently, it has been reported using several mouse models demonstrated that the hypothalamus is important for systemic aging
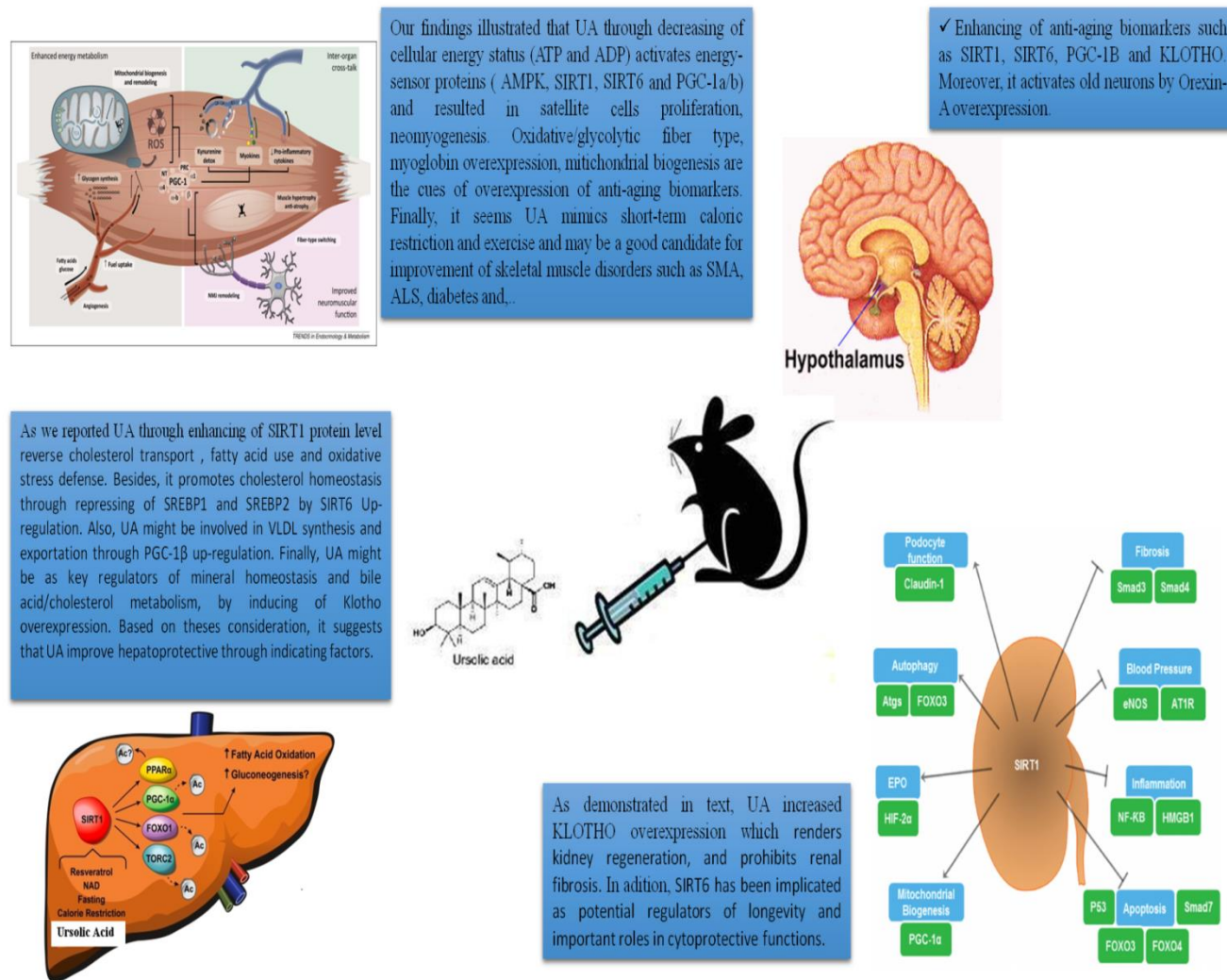
Our findings illustrated that UA through decreasing of cellular energy status (ATP and ADP) activates energy-sensor proteins (AMPK, SIRT1, SIRT6 and PGC-1a/b) and resulted in satellite cells proliferation, neomyogenesis. Oxidative/glycolytic fiber type, myoglobin overexpression, mitichondrial biogenesis are the cues of overexpression of anti-aging biomarkers. Finally, it seems UA mimics short-term caloric restriction and exercise and may be a good candidate for improvement of skeletal muscle disorders such as SMA, ALS, diabetes and,..

✓ Enhancing of anti-aging biomarkers such as SIRT1, SIRT6, PGC-1B and KLOTHO. Moreover, it activates old neurons by Orexin-A overexpression.

As we reported UA through enhancing of SIRT1 protein level reverse cholesterol transport , fatty acid use and oxidative stress defense. Besides, it promotes cholesterol homeostasis through repressing of SREBP1 and SREBP2 by SIRT6 Up-regulation. Also, UA might be involved in VLDL synthesis and exportation through PGC-1β up-regulation. Finally, UA might be as key regulators of mineral homeostasis and bile acid/cholesterol metabolism, by inducing of Klotho overexpression. Based on theses consideration, it suggests that UA improve hepatoprotective through indicating factors.

As demonstrated in text, UA increased KLOTHO overexpression which renders kidney regeneration, and prohibits renal fibrosis. In adition, SIRT6 has been implicated as potential regulators of longevity and important roles in cytoprotective functions.

Fig.1. Ursolic acid rgulates aging through enhancing of anti-aging biomarkers (SIRT1, SIRT6, PGC-1α/β, klotho) and also activates neurons in old animals. As clearly shown in this scheme, ursolic acid obviously regulates aging through up-regulation of master of aging regulators (hypothalamus) and rejuvenating of skeletal muscle by inducing satellite cells proliferation and neomyogenesis. In addition, it protects kidney and liver from age-related diseases. Furthermore, based on recent report UA activates neurons from orexin-A up-regulation.

and lifespan control (Zhang et al., 2013). In addition, it has demonstrated that mice overexpressing SIRT1 in the brain show a delay in ageing and life span extension in males and females, and suggested the importance of hypothalamic SIRT1 in the regulation of aging and longevity in mammals. Noticeably, our recent study illustrated that UA remarkably enhanced SIRT1 protein level in the hypothalamus of old mice (Bahrami and Bakhtiari, 2016). Furthermore, our findings showed that UA increased SIRT6 overexpression as well (Bahrami and Bakhtiari, 2016). Numerous key findings support a critical role for SIRT6 in regulating mammalian longevity (Mostoslavsky et al., 2006). Recently, it has been showed that transgenic mice that overexpress exogenous mouse SIRT6 are protected against the physiological damage caused by diet-induced obesity, including triglyceride and low-density-lipoprotein-associated cholesterol accumulation in the serum, increased body fat and reduced glucose tolerance (Kanfi et al., 2010). Taken together, a novel study proposed that SIRT6 has a main role in mammalian longevity and potential to treat age-related diseases (Kanfi et al., 2012). The klotho gene is a bone-derived hormone that induces negative phosphate balance. With regarding to a potential link between phosphate metabolism and aging, defect in klotho gene expression leads to premat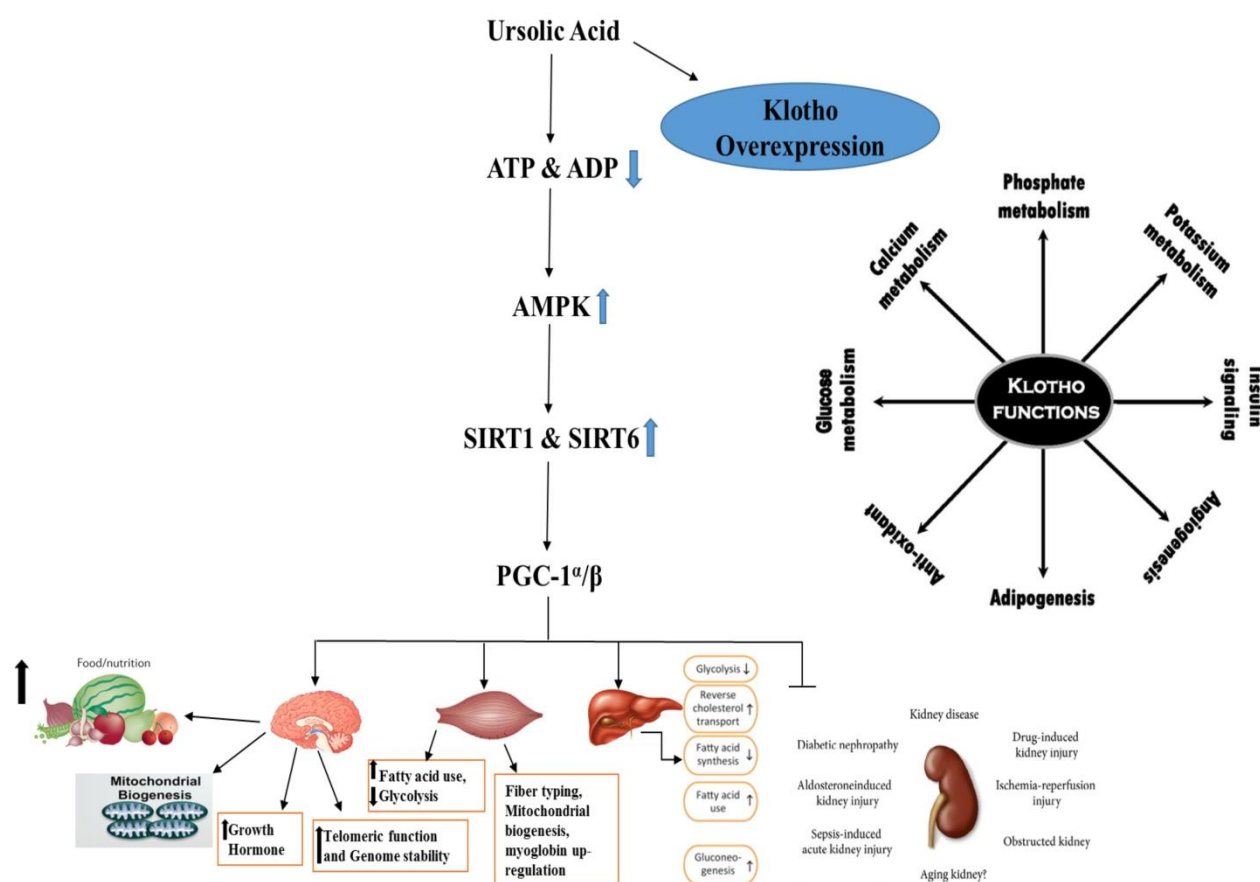ure-aging syndrome in mice (Kuro-o, 2009). With regarding to a potential link between phosphate metabolism and aging, defect in klotho gene expression leads to premature-aging syndrome in mice (Kuro-o, 2009).

Fig.2. Ursolic Acid molecular mechanism. As illustrated in this diargarm UA mechanism effects is through decreasing of cellular energy status (ATP and ADP) which cues to enhancing of energy-sensor proteins (AMPK, sirtuins, PGC-1α/β). As observed in diagram, anti-aging effects of UA emerged through upregulation of these proteins.

Based on previous studies about the worthwhile role of klotho in regulating of aging (Kurosu et al., 2005; Kuro-o, 2011), we sought to know whether UA change the expression of this master anti-aging protein. Outstandingly, the old mice which treated with UA up-regulated klotho in the brain (Bahrami and Bakhtiari, 2016). According to the key role of mitochondria in the pathophysiology of obesity, diabetes, neurodegeneration and aging (Lee and Wei, 2012). PGC1β manages mitochondrial function fatty acid oxidation, hepatic lipogenesis and lipoprotein secretion and cardiac contractile function following stress such as pressure overload hypertrophy and β-adrenergic stimulation (Lelliott et al., 2006). In supporting of these findings, it also showed that ablation of PGC1β neurons seem to be more vulnerable to apoptotic death (Szegezdi et al., 2006) and leads to brain mitochondrial dysfunction (Mahad et al., 2009). Of note, our findings confirmed that UA increased PGC1β protein level and indirectly

involved in mitochondrial function and neuronal health (Bahrami and Bakhtiari, 2016). Therefore, to the best of our knowledge, it seems that UA through enhancing of anti-aging biomarkers (SIRT1 and SIRT6) and PGC1β in the hypothalamus regulates aging-process and attenuates mitochondrial-related diseases. Moreover, an interesting aspect of our study is that it unveils that UA unlike other sirtuins activating compounds such as resveratrol activates neurons and protects them from up-regulation of SIRT1 and orexin-A in the hypothalamus. It seems that UA might be an appropriate candidate in improvement or obstacle of neuron-related disorders including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), sarcopenia, parkinsons and Alzheimer. In regard to the key role of kltho in aging, our data indicate that UA may be on the horizon to forestall diseases of aging.

The emerging role of ursolic acid in the aging

skeletal muscle

To highlight the key role of UA in the systemic regulation of aging and age-related diseases and in the context of skeletal muscle, aging is a complex degenerative process characterized by the diminished capacity for tissue maintenance and gradual decline in organ systems. Skeletal muscle is a key reservoir of amino acids that sustain protein synthesis in other tissue, and limited muscle mass often associated with impaired responses to both stress and critical illness (Wolfe, 2006). The progressive loss of skeletal muscle mass and function in the aged is an important aspect of frailty that is often referred to as sarcopenia (Cruz-Jentoft et al., 2010) and it is largely responsible for the weight loss, weakness and impaired locomotion observed in the elderly. Moreover, one of the hallmarks of skeletal muscle aging is regenerative disability of skeletal muscle (Barberi et al., 2013). There is substantial evidence that show muscle regeneration and growth is driven by satellite cells (Le Grand and Rudnicki, 2007; Bakhtiari, 2016). Satellite cells are quiescent in normal adult muscle and can be activated by muscle damage or other type of stress (Shefer and Yablonka-Reuveni, 2007). It has been reported that concomitant with skeletal muscle satellite cells which depletes with age, the capacity of these cells reduce as well (Le Grand and Rudnicki, 2007). To a large extent, it has been suggested that the proportion of satellite cells found in the muscles of the aged subjects was significantly lower than in young subjects. Two previous studies carried out on muscle aging showed a decrease in the number of muscle fibers during aging (Lexell et al., 1988; Larsson, 1998). Although, recently we showed that UA through satellite cells proliferation promotes new fiber type generation (neomyogenesis) in aged-mice C57Bl/6 (Bakhtiari et al., 2016). It is also believed to counteract muscle weakness, atrophy and frailty by switching fiber typing from glycolytic to oxidative/glycolytic (Bakhtiari et al., 2015a). Concomitant with these studies, our results demonstrated that UA enhances myoglobin level in skeletal muscle (Bakhtiari et al., 2015b). Myoglobin is a well-characterized, cytoplasmic hemoprotein that is expressed primarily in cardiomyocytes and oxidative skeletal muscle fibers (Kanatous and Mammen, 2010). It must be noted that, activation of key transcription factors (myocyte enhancer factor-2,

nuclear factor of activated T-cells and Sp1), co-activators (PGC-1) and intracellular calcium fluxes serve as significant role in modulating myoglobin gene transcription (Lin et al., 2002). In addition, it also reported that PGC-1α plays a main role in regulating myoglobin transcription via a calcineurin/ myocyte enhancer factor-2 dependent mechanism (Lin et al., 2002). In order to verify the significance of these studies, we also observed that UA increases both PGC-1α and β in the old mice (Bakhtiari et al., 2016). PGC-1α, a transcriptional coactivator of peroxisome proliferator- activated receptor-γ encoding key mitochondrial signaling proteins (Fernandez-Marcos and Auwerx, 2011). Previous findings debated in the area of muscle mitochondria and aging, they elucidated that skeletal muscle mitochondrial decline occurs as human's age (Johnson et al., 2013). Interestingly, to approve anti-aging effects of UA, our results declared that UA by decreasing cellular energy charges (ATP and ADP) induces mitochondrial biogenesis through SIRT1 and PGC-1α/β overexpression (Bakhtiari et al., 2016). Previous work had shown that PGC-1α drives the formation of type I and IIA muscle fibers, whereas PGC-1β causes a marked induction of IIX fibers, which are oxidative but have "fast-twitch" properties (Arany et al., 2007). The animal with this type of fibers can run for longer and at higher workloads than wild-type animals (Arany et al., 2007). We next evaluated klotho in skeletal muscle, a multifunctional protein that may be linked to age-associated decline in tissue homeostasis, our novel results unveiled that UA remarkably up-regulates klotho in age-mice. It has been suggested that there is an important link between klotho deficiencies and muscle performance and presents its as a potential inhibitor of age-associated deterioration (Phelps et al., 2013). As mentioned above, one of the hallmarks of skeletal muscle aging is an impairment of their regenerative potential, it has been suggested that SIRT1 inhibits the expression of cell cycle inhibitors and induces its proliferation (Pardo and Boriek, 2011). In this perspective, it seems that UA through decreasing of cellular energy status (ATP and ADP) stimulate AMP-activated protein kinase (AMPK) activation and then leads to SIRT1 overexpression/activation. Up-regulation of these factors causes to satellite cells proliferation and neomyogenesis. Furthermore, UA by increasing PGC-1α/β and klotho has a potential role

in skeletal muscle performance. Taken together, it suggests that UA with mentioned characteristics mimics short-term caloric restriction and fast-exercise training and also has a potential candidate in treatment of skeletal muscle disorders such as ALS, SMA and diabetes.

Ursolic acid and hepatic protection

Normal human ageing occurs with morphological and functional changes in nearly all organ systems and the liver is no exception to this rule. A considerable amount of recent progress has been made in the understanding of how some natural compounds make liver protection. The aging liver appears to preserve its function relatively well. Aging is associated in human liver with morphological changes such as decrease in size attributable to decreased hepatic blood flow and disrupted drug clearance (Anantharaju et al., 2002; Le Couteur and McLean, 1998). The mammalian liver can regenerate and revert to its original size and function following partial resection (Michalopoulos and DeFrances, 1997). This regeneration is mediated by the proliferation of differentiated hepatocytes in the liver, until it regains its original size. However, the rate of regeneration of the liver declines with age (Iakova et al., 2003). One possible explanation is telomere shortening with age, which reduces the capacity of the hepatocytes to proliferate. Nevertheless, a mouse model with deficiency in telomere maintenance showed no difference in liver regeneration (Lazzerini Denchi et al., 2006) due to activation of an alternative mechanism for liver regeneration, resulting in increased cell growth and generation of polyploid cells. Despite this, the mechanism driving liver regeneration and its decline with age remain elusive. Of interests, our data confirmed that UA significantly increases SIRT1 protein level in liver of aged-mice. Certainly, knock outing of SIRT1 in liver makes intervention in fatty acid oxidation, enhanced cellular stress and rising in proinflammatory cytokines (Purushotham et al., 2009). Consistent with these observations, it has been revealed that hepatic SIRT1 may also regulates hepatic cholesterol through deacetylation of sterol response element-binding proteins (SREBPs) (Ponugoti et al., 2010; Walker et al., 2010) and bile acid homeostasis through direct modulation of the liver X receptors and farnesoid X receptor (Li et al., 2007; Kemper et al., 2009). From

these interpretation and accumulating document of sirtuins and their metabolic regulation, we hypothesize that SIRT1 and SIRT6 possess similarities in cellular localization and metabolic functions and question whether UA might change SIRT6 regulation as well. Contemporary, our data validated that UA obviously raises SIRT6 protein level. Previously, it has been shown that SIRT6 protects from hepatic lipid accumulation and hindrances from pathological damage due to diet induced obesity (Kanfi et al., 2010). In supporting of this notion, it has considered that overexpression of SIRT6 brings about reducing the level of SREBP1 and SREBP2 and acts as AMPK activators. Of the many reported AMPK-regulated pathways, it may to highlight glucose homeostasis, cancer and aging (Li et al., 2011; Faubert et al., 2013). Of interest, to support the demonstrated findings, we also declared that UA remarkably increased PGC-1β up-regulation. Recently, one exciting study unveiled that a diet enriched in saturated or trans-fatty acids led to severe stimulation of hepatic PGC-1β expression without altering PGC-1α expression (Lin et al., 2005), they also pointed to this that PGC-1β overexpression in liver stimulate hepatic triglyceride production and secretion, resulting in circulating hypertriglyceridemia and hypercholesterolemia. Conversely, it was previously shown that activation of PGC-1α in liver diminished triglyceride production and secretion (Zhang et al., 2004). Observations using klotho overexpression effects encouraged us to a precise understanding of the possible role of UA in regulating of liver klotho. Therefore, our results demonstrated that UA significantly increases klotho protein level. The klotho gene has a wide range of functions including, anti-aging, anti-apoptotic effects, reducing oxidative stress and inducing the production of nitric oxide (Kuro-o et al., 1997; de Souza Pacheco and Goncalves, 2014). Recently, it has been reported that klotho is a tumor suppressor gene which prohibits the proliferation of liver cancer and promotes cell apoptosis partly due to negative regulation of Wnt/β-catenin signaling pathway (Sun et al., 2015). Finally, UA ameliorates reverse cholesterol transport, fatty acid use and oxidative stress defense through SIRT1 and SIRT6 up-regulation. It involves VLDL synthesis and exportation through PGC-1β up-regulation. Furthermore, UA performs as key regulators of mineral homeostasis and bile

acid/cholesterol metabolism, by inducing of klotho overexpression.

Regulation effects of UA in aging kidney

Even so, there are some common histologic findings and functional changes in the kidney with aging. The biologic mechanism for the changing with age are not well known, but recent identification of senescence genes, the role of hormones and diet may improve our understanding and slow the decline in kidney function (Kitada et al., 2013). With aging, renal blood flow decreases in both human and animal populations (Lamb et al., 2003). The kidney is not a mere excretory organ but also a hormonal source producing several active molecules such as 1,25-(OH)2-vitamin D3, renin, erythropoietin, and klotho (Hu et al., 2013). Klotho is a single-pass transmembrane protein highly expressed in the kidney. Klotho participates in mineral homeostasis via interplay with other calciophosphoregulatory hormones (parathyroid hormone, fibroblast growth factor-23 and 1,25-[OH]2 vitamin D3) in kidney, bone, intestine and parathyroid gland (Kuro-o et al., 1997). Klotho deficiency renders the kidney more vulnerable to acute insults, attenuates kidney regeneration and induces renal fibrosis. In addition to direct renal effects, klotho deficiency also triggers and intensifies disturbed mineral metabolism, secondary hyperparathyroidism, vascular calcification, cardiac hypertrophy and fibrosis. Recently, several studies indicated that nuclear klotho and cytoplasm klotho are also bioactive molecules to protect cells from senescence and apoptosis (Liu et al., 2011; German et al., 2012). According to these descriptions, our *in vivo* study illustrated that UA considerably enhanced klotho protein in kidneys of aged-mice. Moreover, we also showed that UA increases other anti-aging biomarkers in kidneys such as SIRT1, SIRT6 and PGC-1β (Bakhtiari, 2016). In the kidneys, SIRT1 inhibits renal cell apoptosis, inflammation and fibrosis, and regulates lipid metabolism, autophagy, blood pressure and sodium balance (Kitada et al., 2013). Therefore the activation of SIRT1 in the kidney may be a new therapeutic target to increase resistance to many causal factors in the development of renal diseases, including diabetic nephropathy (Kitada et al., 2013). It is also recently reported that a novel kind of communication between two different kidney compartments mediated by SIRT1 which

prevent the above mentioned disorders (Hasegawa et al., 2008; Hasegawa et al., 2010; Hasegawa et al., 2013). Although, SIRT6 has been implicated as potential regulators of longevity and has important roles in cytoprotective functions, their molecular targets, biological functions and possible roles in renoprotection are largely unknown. In debating of mitochondrial biogenesis and kidney diseases, it should be noted that mitochondrial biogenesis and its attendant processes enhance metabolic pathways such as fatty acid oxidation and increase antioxidant defense mechanisms that ameliorate injury from aging, tissue hypoxia and glucose or fatty acid overload, all of which contribute to the pathogenesis of acute and chronic kidney disease (Weinberg, 2011). To validate more beneficial effects of UA, we evaluated master of regulators, PGC-1. Our findings confirmed that UA strikingly up-regulates PGC-1 in kidney of aged-mice (Bakhtiari, 2016). Consequently, the available data indicate that these pathways will be fruitful areas for study in the modification of renal disease.

Concluding comments and future perspective

The considerable increase in human lifespan during the past century meets us with great medical challenges. To confront these challenges, the mechanisms that determine healthy ageing must be understood and controlled. Ursolic acid is a natural compound and for the first time we identified some anti-aging effects of UA with evaluating of prominent anti-aging biomarkers (SIRT1, SIRT6, PGC-1α/β and klotho) in the brain, skeletal muscle, liver and kidneys. Our recent studies demonstrate the regulation of mammalian lifespan by UA which may have important therapeutic implications for age-related diseases. Finally, it suggests that UA through cross-talking between tissues mediates improvement in tissue performance. It exerts functions through decreasing of cellular energy charges, then promotes cellular energy sensors including AMPK, SIRT1 and PGC-1. These biomarkers have beneficial effects in each tissue such as neuroprotective, skeletal muscle rejuvenation, liver protection and prevention of kidney diseases. In addition, we showed that UA activates neurons through orexin-A up-regulation. Finally, it is quite conceivable that supplementation of UA may be a viable therapeutic strategy for treatment of age-associate diseases including diabetes, cancer,

Alzheimer, Parkinson, kidney, liver and skeletal muscle disorders.

Acknowledgments

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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