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ARTICLE

Genes related to maintenance of autophagy and successful aging

Genes relacionados à manutenção da autofagia e envelhecimento bem-sucedido

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ABSTRACT

Considering aging as a phenomenon in which there is a decline in essential processes for cell survival, we investigated the autophagic and proteasome pathways in three different groups: young, older and oldest old male adults. The expression profile of autophagic pathway-related genes was carried out in peripheral blood, and the proteasome quantification was performed in plasma. No significant changes were found in plasma proteasome concentrations or in correlations between proteasome concentrations and ages. However, some autophagy- and/or apoptosis-related genes were differentially expressed. In addition, the network and enrichment analysis showed an interaction between four of the five differentially expressed genes and an association of these genes with the transcriptional process. Considering that the oldest old individuals maintained both the expression of genes linked to the autophagic machinery, and the proteasome levels, when compared with the older group, we concluded that these factors could be considered crucial for successful aging.

Keywords: Autophagy; longevity, gene expression; gene networks; proteasome endopeptidase complex.

RESUMO

Considerando o envelhecimento como um fenômeno em que há um declínio nos processos essenciais a sobrevivência celular, investigamos as vias autofágica e proteassômica em três grupos: jovens, idosos e longevos. O perfil de expressão dos genes relacionados à via autofágica foi analisado em sangue periférico, e a quantificação do proteassoma realizada em plasma. Não foram encontradas alterações significativas nas concentrações plasmáticas de proteassoma ou na correlação entre as concentrações de proteassoma e as idades. No entanto, alguns genes relacionados a autofagia e / ou apoptose foram expressos diferencialmente. Além disso, as análises de rede e de enriquecimento mostraram uma interação entre quatro dos cinco genes diferencialmente expressos e a associação desses ao processo transcricional. Considerando que os indivíduos longevos mantiveram tanto a expressão de genes ligados à maquinaria autofágica, quanto os níveis de proteassoma quando comparados aos idosos, concluímos que esses fatores poderiam ser considerados cruciais para o envelhecimento bem-sucedido.

Palavras-chave: Autofagia; longevidade; expressão gênica; redes regurladoras de genes; complexo de endopeptidases do proteassoma.

Aging is a biological process in which there is a progressive decline in the physiological capacity to respond to environmental stress, and an increase in susceptibility and vulnerability to diseases[1]. Two processes play an important role in homeostasis control and cell survival: ubiquitin-proteasome in redox signaling and the autophagy-lysosome pathway in damaged protein degradation[2].

The ubiquitin-proteasome is a multicatalytic ATP-dependent degradation system found in both cytoplasm and cell nucleus[3,4,5]. This system is related to the degradation of normal or abnormal proteins, and some studies have shown decreased proteasomic activity during aging in different human tissues such as muscle and epidermis[6,7,8].

A study developed by Lee et al.[9] showed a reversion in decreased proteasome gene expression in the skeletal muscle of mice submitted to caloric restriction, indicating that this intervention may contribute to the prevention of aging by increasing degradation of damaged proteins. Recently, the 20S proteasome was found in human blood and showed a proteolytic activity[10,11,12]. Although the origin of these circulating proteasomes is still unknown, some studies have reported their increased concentrations in pathological conditions

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such as cancer[13,14], and suggested a correlation between proteasome concentrations and health status[10,15,16].

Several diseases related to aging show accumulation of oxidized proteins and the failure of autophagic pathways is suggested as a possible cause[17]. The autophagy-lysosome pathway is a cytoplasmic-restricted degradation system, related to the degradation of organelles, proteins and protein aggregates[18]. Under adequate levels of nutrients, growth factors and reactive oxygen species, autophagy is at basal levels (constitutive) with normal protein biosynthesis. However, autophagy can be induced by a stressor and the production of proteins interrupted[19].

As the molecular characterization of the autophagic machinery may allow the development of tools for a better physiological and molecular evaluation of successful aging, our objective was to quantify the expression of genes involved in autophagic machinery regulation in young, older and oldest old adult male individuals.

METHODS

Volunteers

The individuals selected for this study were previously recruited by the Department of Preventive Medicine and Discipline of Geriatrics and Gerontology of the Universidade Federal de São Paulo for a different study but the samples were not fully used. All volunteers signed a free and informed consent form. For the current study, they signed an authorization for sample use, following norms determined by the Research Ethics Committee of the Universidade Federal de São Paulo, which approved the study (# 451631/2013). The sample consisted of male volunteers, distributed into three groups: individuals aged between 20 and 30 years (young group, n = 15), individuals aged between 60 and 70 years (older group, n = 13) and individuals between 85 and 105 years old (oldest old group, n = 10). Individuals with neoplasias or severe unmanaged diseases, such as heart diseases, gastrointestinal diseases, type 2 diabetes, or with neurological and psychiatric antecedents were excluded.

Samples previously collected

Peripheral blood was collected in EDTA tubes, centrifuged at 3,000 rpm for 10 minutes and the separated plasma stored at -20°C. In addition, 5 mL of blood was collected in specific tubes (PaxGene RNA collection tubes – PreAnalytiX, Switzerland) for total RNA extraction using the PaxGene kit (PaxGene blood RNA isolation kit – PreAnalytiX, Switzerland). After verification of integrity and purity, total RNA was stored at -80°C.

Proteasome

To perform proteasome quantification in plasma, we used enzyme-linked immunosorbent assay – Proteasome ELISA Kit (Enzo Life Sciences, BML-PW0575, EUA), which employs specific antibodies for the 20S proteasome subunit. The product absorbance was detected using the SpectraMax M2 apparatus (Molecular Devices, USA).

Gene expression

Total RNA was quantified using the NanoDrop 8000 (Thermo Scientific, USA). For complementary DNA synthesis, we used the RT2 First Strand Kit (QIAGEN, Germany) plus 625 ng of RNA. Cycling parameters comprised a holding stage at 42ºC for 15 minutes, followed by inactivation at 95°C for 15 minutes. The expression profile of 84 autophagic pathway-related genes was analyzed in peripheral blood RNA samples using the Superarray-RT2 Profiler™ PCR Array System (QIAGEN, Germany – PAHS-084ZD-24) in the 7500 PCR Real-Time System (Applied Biosystems, USA). In addition, *ACTB*, *B2M*, *GAPDH*, *HPRT1* and *RPLP0* genes were evaluated as an endogenous control. Thermal cycling conditions comprised an initial denaturation at 95°C for 15 seconds and annealing and extension at 60°C for one minute (Table 1).

Gene expression quantification was obtained using ΔCT calculation, and the endogenous control CT was obtained from the arithmetic mean of two endogenous controls that showed a lower variation between groups (standard deviation > 0.1). Then, the relative gene expression was calculated by CT comparative method (ΔΔCT) using the following formula: FC = 2-ΔΔCT = 2-(ΔCT interest group - ΔCT reference group). For better visualization of variation, data were presented by fold regulation (FR) (if FC was greater than 1, FR = FC, if the HR was less than 1, the FR = -(1/FC)), which represents the number of times a gene is expressed in one group in relation to the other. Both the older and oldest old groups were compared with the young group (reference group).

Network and enrichment analysis

To investigate interactions and pathways shared by differentially expressed genes, two online software applications were used: GeneMANIA[20] (www.genemania.org]) and Enrichr[21] (http://amp.pharm.mssm.edu/Enrichr). GeneMANIA allows genes with shared or functionally similar properties to be identified. These analyses may be performed with genes of interest or with 20, 50 or 100 other genes in the interaction. In the present study, we used 20 genes. Enrichr enables enrichment analysis, where genes of interest are searched and compared in databases to verify possible pathways and over-represented cellular processes in which they may participate.

Statistical analysis

The normality of data was analyzed with Shapiro-Wilk's test and, when necessary, normalized using the Z-score. Data was compared using one-way analysis of variance (ANOVA). We used the Pearson's correlation test to compare age and proteasome levels in each age group. Data are presented as mean ± standard error. The level of significance was set at $p \leq 0.05$. However, the p value was corrected by the Benjamini-Hochberg

Table 1: Genes from autophagic machinery investigated by the Superarray - RT2 Profiler ™ PCR Array System (PAHS-084Z).

Position	GeneBank	Symbol	Description
A01	NM_005163	AKT1	V-akt murine thymoma viral oncogene homolog 1
A02	NM_017749	AMBRA1	Autophagy/beclin-1 regulator 1
A03	NM_000484	APP	Amyloid beta (A4) precursor protein
A04	NM_031482	ATG10	ATG10 autophagy related 10 homolog (S. cerevisiae)
A05	NM_004707	ATG12	ATG12 autophagy related 12 homolog (S. cerevisiae)
A06	NM_017974	ATG16L1	ATG16 autophagy related 16-like 1 (S. cerevisiae)
A07	NM_033388	ATG16L2	ATG16 autophagy related 16-like 2 (S. cerevisiae)
A08	NM_022488	ATG3	ATG3 autophagy related 3 homolog (S. cerevisiae)
A09	NM_052936	ATG4A	ATG4 autophagy related 4 homolog A (S. cerevisiae)
A10	NM_178326	ATG4B	ATG4 autophagy related 4 homolog B (S. cerevisiae)
A11	NM_178221	ATG4C	ATG4 autophagy related 4 homolog C (S. cerevisiae)
A12	NM_032885	ATG4D	ATG4 autophagy related 4 homolog D (S. cerevisiae)
B01	NM_004849	ATG5	ATG5 autophagy related 5 homolog (S. cerevisiae)
B02	NM_006395	ATG7	ATG7 autophagy related 7 homolog (S. cerevisiae)
B03	NM_024085	ATG9A	ATG9 autophagy related 9 homolog A (S. cerevisiae)
B04	NM_173681	ATG9B	ATG9 autophagy related 9 homolog B (S. cerevisiae)
B05	NM_004322	BAD	BCL2-associated agonist of cell death
B06	NM_001188	BAK1	BCL2-antagonist/killer 1
B07	NM_004324	BAX	BCL2-associated X protein
B08	NM_000633	BCL2	B-cell CLL/lymphoma 2
B09	NM_138578	BCL2L1	BCL2-like 1
B10	NM_003766	BECN1	Beclin 1. autophagy related
B11	NM_001196	BID	BH3 interacting domain death agonist
B12	NM_004052	BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
C01	NM_004346	CASP3	Caspase 3. apoptosis-related cysteine peptidase
C02	NM_001228	CASP8	Caspase 8. apoptosis-related cysteine peptidase
C03	NM_004064	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27. Kip1)
C04	NM_000077	CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma. p16. inhibits CDK4)
C05	NM_000086	CLN3	Ceroid-lipofuscinosis. neuronal 3
C06	NM_001908	CTSB	Cathepsin B
C07	NM_001909	CTSD	Cathepsin D
C08	NM_004079	CTSS	Cathepsin S
C09	NM_003467	CXCR4	Chemokine (C-X-C motif) receptor 4
C10	NM_004938	DAPK1	Death-associated protein kinase 1
C11	NM_018370	DRAM1	DNA-damage regulated autophagy modulator 1
C12	NM_178454	DRAM2	DNA-damage regulated autophagy modulator 2
D01	NM_004836	EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 3
D02	NM_182917	EIF4G1	Eukaryotic translation initiation factor 4 gamma. 1
D03	NM_000125	ESR1	Estrogen receptor 1
D04	NM_003824	FADD	Fas (TNFRSF6)-associated via death domain
D05	NM_000043	FAS	Fas (TNF receptor superfamily. member 6)
D06	NM_000152	GAA	Glucosidase. alpha; acid
D07	NM_007278	GABARAP	GABA(A) receptor-associated protein
D08	NM_031412	GABARAPL1	GABA(A) receptor-associated protein like 1
D09	NM_007285	GABARAPL2	GABA(A) receptor-associated protein-like 2
D10	NM_004964	HDAC1	Histone deacetylase 1

Continue

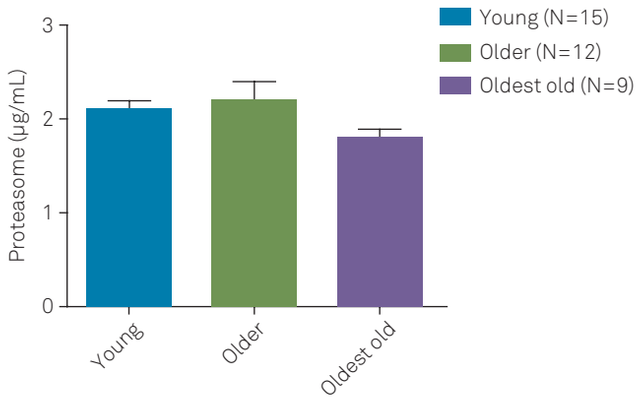
Continuation

D11	NM_006044	HDAC6	Histone deacetylase 6
D12	NM_004712	HGS	Hepatocyte growth factor-regulated tyrosine kinase substrate
E01	NM_001017963	HSP90AA1	Heat shock protein 90kDa alpha (cytosolic). class A member 1
E02	NM_006597	HSPA8	Heat shock 70kDa protein 8
E03	NM_002111	HTT	Huntingtin
E04	NM_000619	IFNG	Interferon. gamma
E05	NM_000618	IGF1	Insulin-like growth factor 1 (somatomedin C)
E06	NM_000207	INS	Insulin
E07	NM_001145805	IRGM	Immunity-related GTPase family. M
E08	NM_005561	LAMP1	Lysosomal-associated membrane protein 1
E09	NM_181509	MAP1LC3A	Microtubule-associated protein 1 light chain 3 alpha
E10	NM_022818	MAP1LC3B	Microtubule-associated protein 1 light chain 3 beta
E11	NM_001315	MAPK14	Mitogen-activated protein kinase 14
E12	NM_002750	MAPK8	Mitogen-activated protein kinase 8
F01	NM_004958	MTOR	Mechanistic target of rapamycin (serine/threonine kinase)
F02	NM_003998	NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
F03	NM_000271	NPC1	Niemann-Pick disease. type C1
F04	NM_002647	PIK3C3	Phosphoinositide-3-kinase. class 3
F05	NM_002649	PIK3CG	Phosphoinositide-3-kinase. catalytic. gamma polypeptide
F06	NM_014602	PIK3R4	Phosphoinositide-3-kinase. regulatory subunit 4
F07	NM_006251	PRKAA1	Protein kinase. AMP-activated. alpha 1 catalytic subunit
F08	NM_000314	PTEN	Phosphatase and tensin homolog
F09	NM_130781	RAB24	RAB24. member RAS oncogene family
F10	NM_000321	RB1	Retinoblastoma 1
F11	NM_005873	RGS19	Regulator of G-protein signaling 19
F12	NM_003161	RPS6KB1	Ribosomal protein S6 kinase. 70kDa. polypeptide 1
G01	NM_000345	SNCA	Synuclein. alpha (non A4 component of amyloid precursor)
G02	NM_003900	SQSTM1	Sequestosome 1
G03	NM_000660	TGFB1	Transforming growth factor. beta 1
G04	NM_004613	TGM2	Transglutaminase 2 (C polypeptide. protein-glutamine-gamma-glutamyltransferase)
G05	NM_153015	TMEM74	Transmembrane protein 74
G06	NM_000594	TNF	Tumor necrosis factor
G07	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily. member 10
G08	NM_000546	TP53	Tumor protein p53
G09	NM_003565	ULK1	Unc-51-like kinase 1 (C. elegans)
G10	NM_014683	ULK2	Unc-51-like kinase 2 (C. elegans)
G11	NM_003369	UVRAG	UV radiation resistance associated gene
G12	NM_017983	WIPI1	WD repeat domain. phosphoinositide interacting 1
H01	NM_001101	ACTB	Actin. beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein. large. P0
H06	SA_00105	HGDC	Human Genomic DNA Contamination
H07	SA_00104	RTC	Reverse Transcription Control
H08	SA_00104	RTC	Reverse Transcription Control
H09	SA_00104	RTC	Reverse Transcription Control
H10	SA_00103	PPC	Positive PCR Control
H11	SA_00103	PPC	Positive PCR Control
H12	SA_00103	PPC	Positive PCR Control

method (pBH) for gene expression analyses. Fold regulation (FR) values greater than 1.50 (genes with increased expression) or less than -1.50 (genes with decreased expression) were used to select differentially expressed genes, and to exclude those potentially subject to methodological noise. Thus, the differentially expressed genes were those included in one of the following conditions: 1) pBH ≤ 0.05, independent of the FR value or 2) p ≤ 0.05 and FR ≥ 1.50 or FR ≤ -1.50.

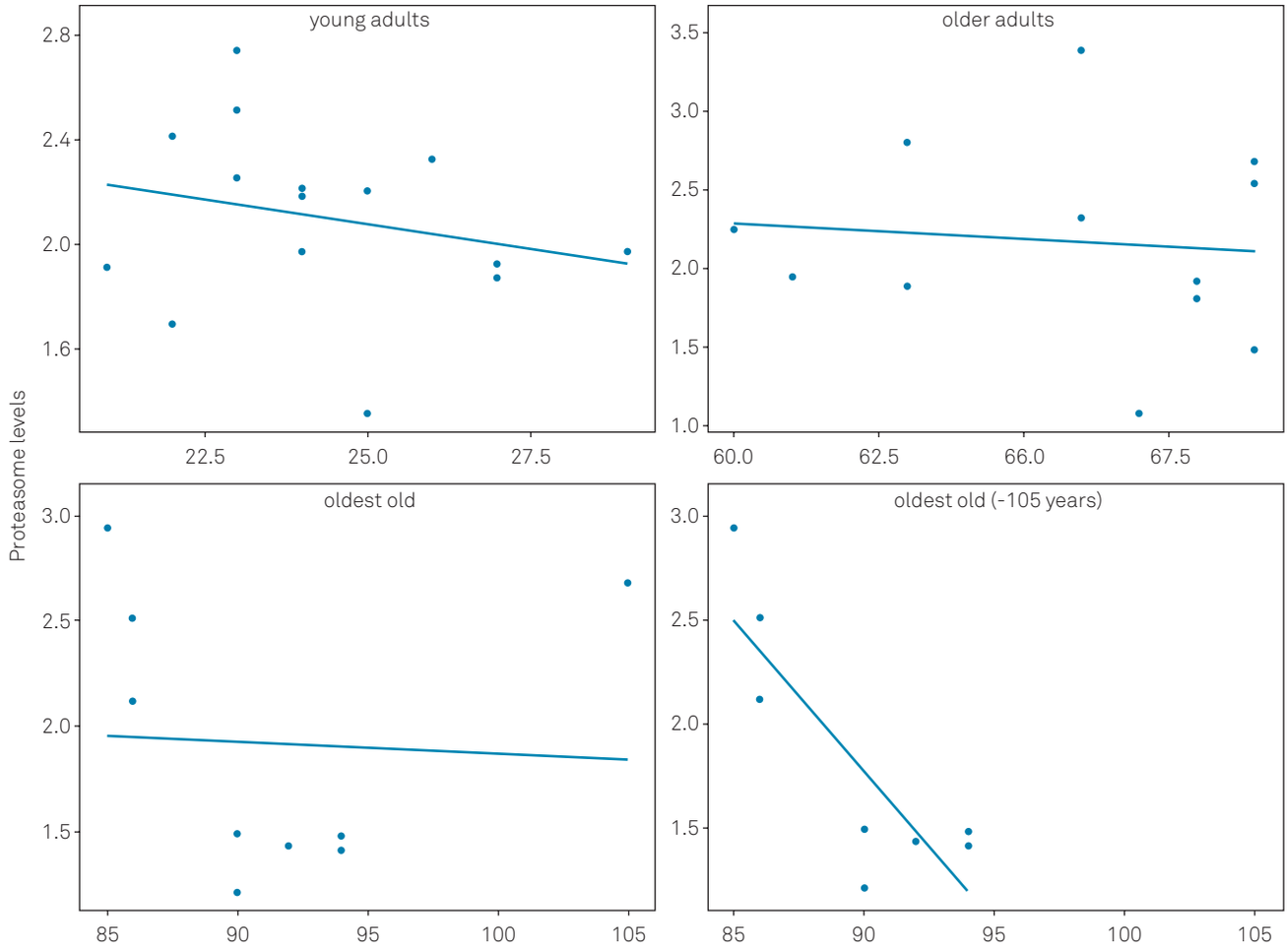
RESULTS

Autophagic pathway gene expression and proteasome levels were evaluated in the individuals from three different age groups (mean ± standard deviation): young, 24.3 ± 2.2 years: older, 65.5 ± 3.0 years; and oldest old, 91.9 ± 6.1 years ($F_{(2,35)} = 999.95$; $p < 0.001$). The mean and standard deviations of body mass index were 24.04 ± 2.74 in the young group, 25.87 ± 3.56 in the older group, and 24.94 ± 3.55 in oldest old group ($F_{(2,35)} = 1.10$; $p = 0.333$). No difference was observed in proteasome levels between the three age groups (ANOVA; $F_{(2,34)} = 0.619$ and $p = 0.545$; Figure 1). Additionally, plasma proteasome levels were not related to the individuals' ages in each group (Figure 2). However, when the oldest individual (105 years) was excluded from the oldest old group analysis, a statistically significant correlation was observed (Figure 2D).



ANOVA. $F_{(2,34)} = 0.619$ and $p = 0.545$. Data are presented as the mean ± standard error.

Figure 1. Proteasome levels in young, older and oldest old groups.



Young: n = 15, r = -0.240, p = 0.389; Older: n = 12, r = -0.097, p = 0.765; Oldest old: n = 9, r = -0.052, p = 0.894.

Figure 2. Pearson's correlation between proteasome levels and age in the young, older and oldest old groups.

Regarding gene expression, from the 84 genes linked to autophagic machinery, only five were differentially expressed according to the adopted criteria: *ATG4C*, *BCL2L1*, *EIF2AK3*, *EIF4G1* and *TP53* (Table 2). The *ATG4C* gene was significantly less expressed in the oldest old group when compared with the young group (1.91-fold decrease); in addition, there was also a difference in the older group when compared with the oldest old (1.47-fold increase; p = 0.031). The *BCL2L1* gene was significantly more expressed in the oldest old when compared with the young group (increase of 1.91 times). The *EIF2AK3* gene was significantly less expressed in the older group (1.46-fold decrease), as well as in oldest old individuals when compared with the young group (1.44-fold decrease). The *EIF4G1* gene was significantly less expressed in the older and oldest old when compared with the young group (decrease of 1.47 and 1.32 times, respectively). The *TP53* gene was significantly less expressed in the older and oldest old when compared with the young group (decrease of 1.57 and 1.66-fold, respectively).

In the network analysis, we observed that from the five differentially expressed genes, only two showed evidence of some interaction — *TP53* and *BCL2L1* (Figure 3). When the other 20 genes were added, we observed that four of the five genes showed some type of interaction, the exception being *ATG4C* (Figure 4). The enrichment analysis was divided

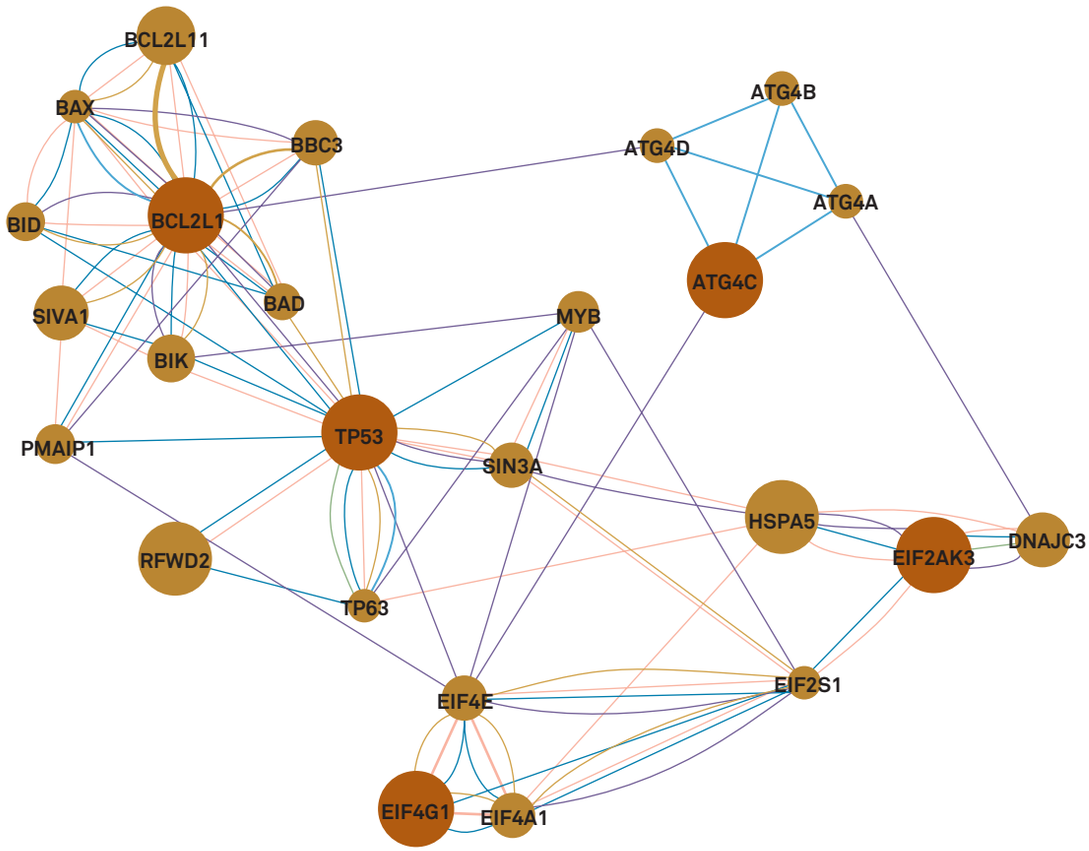
Table 2. Differentially expressed genes in older and oldest old in relation the young group.

Genes	pBH	p	Older (N=13)		oldest old (N=10)	
			FR	pa	FR	pb
ATG4C	0.008	<0.001	-1.30	0.136	-1.91	<0.001
BCL2L1	0.277	0.030	1.11	1.000	1.91	0.033
EIF2AK3	0.026	0.001	-1.46	0.003	-1.44	0.009
EIF4G1	0.011	<0.001	-1.47	<0.001	-1.32	0.021
TP53	0.002	<0.001	-1.57	<0.001	-1.66	<0.001

pBH: p value corrected by Benjamini-Hochberg method; FR: fold regulation in relation to the young group; p: referent ANOVA values from $2^{-\Delta CT}$; p^a: t test results from $2^{-\Delta CT}$ values between the older and young groups; p^b: t test results from $2^{-\Delta CT}$ values between the oldest old and young groups.

Blue line: interaction by pathways; pink line: physical interaction; orange line: prediction of protein-protein interaction.

Figure 3. Interactions between the five differentially expressed genes among the young, older and oldest old groups.



Light blue line: interaction by pathways; pink line: physical interaction; purple line: co-expression; green line: genetic interaction; dark blue line: co-localization; brown line: shared protein domains; orange line: protein-protein interaction prediction.

Figure 4. Interaction of five differentially expressed genes in the young, older and oldest old groups after inclusion of 20 genes in the network analysis.

into two stages: the first was done with the five differentially expressed genes, and the second with the differentially expressed genes plus the genes that showed the most frequent pathways in the network analysis (*HSPA5*, *SIN3A* and *EIF2S1*). In the first step, the following databases were used: TRANSFAC and JASPAR PWMs, ENCODE TF ChIP-seq 2015, ESCAPE, ENCODE TF ChIP-seq and GO Biological Process 2013. The databases used in the second stage were: ChEA, TRANSFAC and JASPAR PWMs, ENCODE TF ChIP-seq 2015, transcription factor PPIs, ESCAPE, ENCODE TF ChIP-seq. All databases used in the first and second stages showed direct or indirect linkage of the genes analyzed with the transcription process.

DISCUSSION

The accumulation of macromolecules and damaged organelles is one of the most predominant alterations found in aged cells, and the main cause is related to a deficient autophagic process[22]. Studies in *C. elegans* and *D. melanogaster* have shown that the loss of function of autophagy genes is related to an accumulation of damaged organelles and proteins, accelerated aging and shortened life span[23,24,25]. To evaluate the contribution of the autophagic machinery in successful aging, we quantified the expression of 84 genes related to the autophagic pathway in young, older and oldest old individuals; five presented with differential expression between the studied groups: *ATG4C*, *BCL2L1*, *TP53*, *EIF2AK3* and *EIF4G1*.

The *ATG4C* encodes a protein with protease activity involved in autophagic vacuole formation. However, studies suggest that this protein is not essential to generate the basal level of autophagy required, since knockout mice for the *ATG4C* gene exhibit normal development[24]. In contrast, knockouts for this gene are more likely to develop fibrosarcoma when exposed to carcinogenic chemicals compared with wild-type animals[26]. The lower expression of *ATG4C* observed in the oldest old people group does not suggest lower autophagic activity per se, but may contribute to a higher risk of these individuals developing tumors, a condition that could be related to aging. On the other hand, the increased expression of *BCL2L1* observed in the older and oldest old groups indicates that autophagy levels decrease during aging[27]. The *BCL2L1* is a co-regulator of autophagy and apoptosis and proteins from the BCL-2 family may also interact with p53 in the induction of autophagy. P53 exhibits tumor suppressor activity and the ability to control autophagic processes and cellular senescence[28,29]. In the current study, there was decreased *TP53* expression in both the oldest old and older groups in relation to the young group, suggesting that the autophagic process decreases with increased age. In addition, decreased expression of *EIF2AK3* and *EIF4G1* in both the oldest old and older individuals reflects the body's declining ability to maintain reticulum homeostasis and cellular processes with increasing age[19,30]. The *EIF2AK3* and *EIF4G1* proteins, respectively, are associated with endoplasmic reticulum homeostasis and the initiation of translation of mRNAs related to mitochondrial activity and cellular bioenergetics[19,30].

Studies have suggested that proteasome activity declines during cellular senescence and aging in both animal models and humans[31]. However, a study performed by Chondrogianni and colleagues showed similar functional proteasomes in human fibroblasts cultures from centenarian and young donors[8]. In the current study, we evaluated, for the first time, the plasmatic proteasome levels in the young, older and oldest old groups and we did not observe a significant difference between them. Although there is no evidence that plasmatic proteasome concentrations reflect the intracellular proteasome activity, we hypothesized that the similarity of plasma proteasome concentrations between the groups found in our samples could be one of the factors contributing to the longevity in the oldest old group. In fact, we previously observed that these same oldest old individuals had a more favorable lipid profile compared with the other groups[32]. An increase in *SIRT2* expression in the oldest old people was also observed when compared with the young group (unpublished data). The increase in *SIRT2* seems to contribute to the promotion of longevity by increasing levels of autophagy[33]. More recently, several studies have shown the impact of caloric restriction on sirtuin levels, which in turn act on autophagic pathways and contribute to increased life expectancy[34]. In the network analysis of differentially expressed genes, we identified interactions between the *TP53* and *BCL2L1* genes, which was expected, as several studies have shown the promotion of autophagy by the interaction of TP53 with the Bcl-2 family proteins[35-37]. However, when we added 20 other genes to this network, four of the five differentially expressed genes had some type of interaction, with *ATG4C* being the exception (Figure 4). The interaction between the four genes is related to the regulation of transcription, an extremely important process for cell functioning[38]. During the aging process, some genes have increased expression, such as those related to cell adhesion and immune response[39], while others have decreased expression, such as genes that participate in lipid metabolism[39] and those involved in the electron transport chain[40,41].

In conclusion, the *ATG4C*, *BCL2L1*, *TP53*, *EIF2AK3* and *EIF4G1* genes differed preferentially when comparing the oldest old and older with the young group, suggesting that autophagy and some processes like maintenance of metabolism and control of gene expression are impaired when the individual ages. On the other hand, the similarity in the expression pattern observed between the older and oldest old suggests that the maintenance of these pathways related to homeostasis plays an important role in increasing life expectancy. In general, these findings, together with the maintenance of proteasome levels observed in the oldest old individuals, point to the maintenance of autophagy as a crucial factor for longevity.

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