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SIRT1, IGFBP-3 and CAV1 Promoter DNA Methylation in Aging

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Abstract

The human genome is a fragile and highly conserved structure that accumulates a wide range of damaging alterations with age. Long-term alterations in the transcriptional potential of a cell are heritable and these changes are called epigenetics. The most studied alteration is the DNA methylation, which can regulate gene expression, often blocking gene transcription. The relationship between epigenetics and aging was proposed many years ago, and a decrease in the genomic global DNA methylation with age was largely observed. In this study we describe the methylation status of *SIRT1*, *IGFBP-3* and *CAV1* in a young and an older group of healthy individuals by methylation-specific PCR. *SIRT1*and *IGFBP-3* promoter methylation frequency was significantly higher in the older than in the young adult group. Moreover, when combining promoter methylation status of several genes we observed that the total number of methylated promoters more than 85% of older individuals presented 4 promoters against only 46% of young adults. These findings may suggest that with increasing age there is an epigenetic switching to gene repression. The understanding of age-related genes methylation status can lead to a better comprehension of the role of epigenetic variation in the aging process.

Keywords: Aging; DNA methylation; Epigenetic; *SIRT1*; *CAV1*; *IGFBP-3*

Introduction

Epigenetic is defined as heritable changes in gene activity and expression [1]. The most known epigenetic modifications are DNA methylation and histone post-transcriptional modifications. DNA methylation consists of the addition of a methyl group to the aromatic ring of 5-carbon of the cytosine ring of a CpG dinucleotide. This dinucleotide is concentrated in particular gene promoters, called CpG islands, where it can regulate gene expression, often blocking transcription when the methyl group is present [1].

The human genome is a fragile and highly conserved structure that accumulates a wide range of damaging alterations with age, despite continuous surveillance and repair [2]. In addition to this global hypomethylation, a number of specific loci have been described as hypermethylated during aging [1]. Interesting, this events are known epigenetic alterations in cancer, which suggests that the accumulation of epigenetic alterations during aging may directly contribute to malignant transformation [3].

A recent paper describes an appealing predictor of age that estimates the DNA methylation age of most tissues. The author proposes that DNA methylation age measures the cumulative effect of an epigenetic maintenance system very useful for developmental biology, cancer and aging research [4].

In this study, we selected three genes, *SIRT1*, *IGFBP-3* and *CAV-1*, to evaluate DNA promoter methylation in the aging process. To our knowledge, this is the first study of DNA promoter methylation in these genes in normal aging process.

Material and Methods

Samples

Methylation status of *SIRT1*, *IGFBP-3* and *CAV-1* were evaluated in peripheral blood samples of two groups: 1) Older Adults, including about 50 healthy older adults, with exclusion criteria for depression, stroke, dementia and low cognitive levels using Mini-Mental State Exam (MMSE), 2) Younger Adults including about 60 healthy young adult volunteers. Mean age for groups were 70.06 ± 9.644 years and 21.46 ± 2.482 years, respectively. All older individuals were selected at Neurology Department of Federal University of São Paulo. All individuals signed a written informed consent form.

DNA extraction and Methylation Specific PCR (MSP)

DNA extraction was performed using the QIAamp DNA Blood Midi Kit (Qiagen). To evaluate methylation status, genomic DNA (200 ng) of all samples underwent bisulfite modification using EpiTect Bisulfite kit (Qiagen, Germany) according to the manufacturer's instructions. Specific primers for MSP, located within the genes' promoter, are described in Table 1. Briefly, PCR reaction was carried out in a 25 µL volume with 200 µmol/L of $MgCl_2$, 100 ng of DNA, 200 pmol/L of primers and 1.25 units of Taq (LGC, Brazil). After initial denaturating for 5 min at 94ºC, 40 cycles at 94ºC for 45 s, at respectively temperature (Table 1) for 45 s, and at 72ºC for 30 s were carried out, followed by a final extension for 5 min at 72ºC. Results were scored when there was a clear and visible band on the electrophoresis gel with the methylated and unmethylated primers [5] (Figure 1).

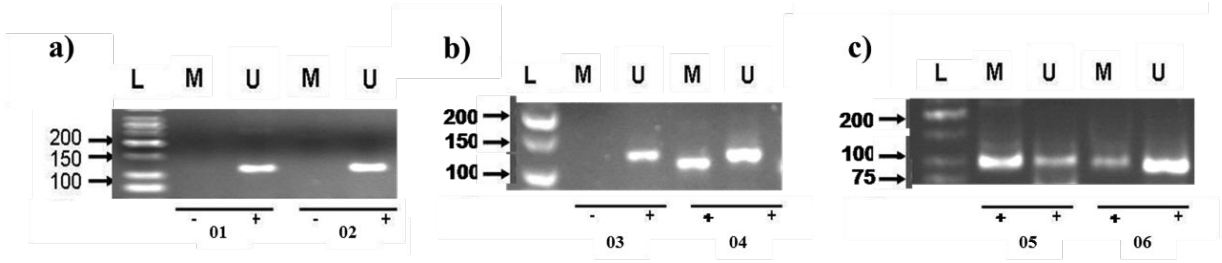

Figure 1: Methylation analysis by MSP of gene promoters: a) *SIRT1* showing unmethylated samples (01 and 02) b) *IGFBP-3* showing unmethylated (03 and methylated samples (04) c) *CAV1* showing methylated samples (05 and 06). L: size marker; M: methylated; U: unmethylated

Gene	Sense	Anti-sense	Product	Tm (ºC)
SIRT1	M- GGCGAATTTGGTTGTATTATACG U- GGGGTGAATTTGGTTGTATTATATG	M- GAACGAAAACTATTACGTCTACCG U- AAACAAAAACTATTACATCTACCACT	110bp 112bp	62.2
IGFBP-3	M-TTTCGGTTTTTATATAGCGGTC U-TTATTTTGGTTTTTATATAGTGGTT	M-AAAAAACGACTAATCCTCAACG U-AACAAAAAACAACTAATCCTCAACA	84pb 90pb	54.1
CAV-1	M-TTTCGGGACGTTTTTCGGTGGT U-GAAAATATTTGTTTTTTTTGGGAT	M-TAAAAACGTTTCTCCCGCGCTA U-ACAAATAAAAACATTTCTCCCACA	96bp 116bp	57.8

Table 1: Primers sequences, product size and annealing temperature for *SIRT1, IGFBP-3* and *CAV-1* used in methylation specific PCR

Statistical analyses

Statistical analyses were performed using the $\chi 2$ test or Fisher's exact test to assess associations between methylation status and the two groups. P values less than 0.05 were considered significant.

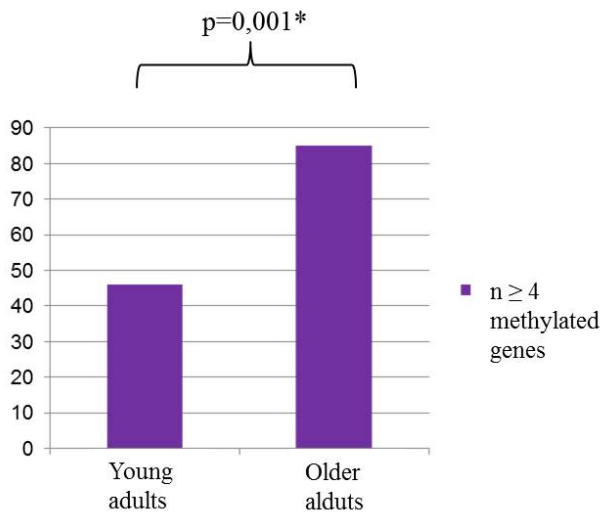

Figure 2: Graph showing percentage of young adults and older adults groups with 4 or more methylated promoter genes, among the seven age-related genes studied by our group

	Unmethylated (%)	Methylated (%)	p
SIRT1			0.024*
Young Adults	58 (98.3%)	1 (1.7%)	
Older Adults	44 (86.3%)	7 (13.7%)	
IGFBP-3			0.022*
Young Adults	19 (31.1%)	42 (68.9%)	
Older Adults	6 (12%)	44 (88%)	
CAV1			0.288
Young Adults	6 (10.3%)	52 (89.7%)	
Older Adults	2 (4.2%)	46 (95.8%)	

Table 2: Methylation frequency of *SIRT1, IGFBP-3* and *CAV1* promoter by groups, n (%)

Results

SIRT1 promoter methylation was more frequently observed in the older than in the young adult group (p=0.024) (Table 2). *IGFBP-3* also presented promoter hypermethylation in 88% of older group and in 68.9% of younger group (p=0.022).

The methylation pattern of *CAV-1* did not differ between groups (p=0.288) (Table 2). Also, no statistical differences were observed for gender for *IGFBP-3* (p=0.813), *SIRT1* (p=1) and *CAV1* (p=0.256).

Moreover, when combining *SIRT1, IGFBP3* and *CAV1* methylation analyses with previous data published by our group by Silva et al. [6], that studied methylation status of *SIRT3, SMARCA5, hTERT* and *CDH1* in the same sample, we observed that the total numbers of methylated promoters are significantly higher in the older group than in the young control group. More than 85% of aged individuals presented 4 or more methylated gene promoters against only 46% of young adults (p=0.001) (Figure 2).

Discussion

Aging process is associated with several physiologic alterations that occur overtime. These alterations may appear in molecular level, in both genetic and epigenetic pathway depending on genotype, environment and stochastic factors.

DNA methylation pattern of CpG islands is somatic heritable and can silence gene expression. Wa number of specific loci become hypermethylated with aging process [7]. Therefore, genes that have an important role in several cell processes can be susceptible to these alterations.

Sirtuin is a NAD dependent deacetylases family conserved in many organisms, from yeast to humans [8]. SIRT1 has a nuclear location, playing a role in the deacetylation of histone and several non-histone targets, including genes involved in cell cycle, survival, stress response and apoptosis [9].

SIRT1 polymorphisms have been related to healthy aging [10] but, to our knowledge, this is the first study to observe an association between higher promoter methylation in aged group when compared to young group. This fact suggests an epigenetic regulation by DNA methylation and higher *SIRT1* expression in young individuals, probably decreasing overtime. *SIRT1* activity to induce cell cycle arrest and DNA repair, as well as apoptosis and senescence may reflect the aging cell differences [11-13]. In Alzheimer's disease, our group observed no differences in DNA methylation levels among elderly and AD groups [14], despite that, we show here differences between healthy young and aged individuals.

IGFBP-3 transcriptional regulation may be mediated by epigenetic modifications, such as methylation [15]. Our results show a higher promoter DNA methylation in aged group when compared to young group; this result is consistent with the CpG island methylation increasing with age [16]. This data support another study that has shown lower DNA methylation in children and young adults [17]. *IGFBP-3* is the major binding protein of IGF-1, growth inhibitor and pro-apoptotic factor. The deletion of the studied CpG island appears to be important for decrease mRNA expression in senescent cell [18], therefore, the increased hypermethylation observed might also have an influence in *IGBFP-3* expression.

In a previous study by our group in gastric tissue, we have not observed any differences of methylation with age [19]. This finding may be due to tissue-specific epigenetic regulation [20]. Our data may reflect a lower level of IGFBP-3 in serum, therefore its function as endocrine cell growth [21]. Controversially, a study in cellular senescence in human fibroblasts has reported increased mRNA levels of IGBFP-3 [22].

CAV1 is a candidate for tumor suppressor and has been mapped in chromosome 7q31.1, a region commonly deleted in cancer [23]. The gene product, the Caveolin-1 (CAV1) is an integral membrane protein, principal component of caveolae [24]. CAV1 interacts with signaling molecules via membrane-proximal domain, and has been also implicated in regulation of activities and quantitation of cross-talk between distinct signaling cascades [25].

Methylation of the *CAV1* gene promoter has been described in different types of cancer, such as prostate [26], breast [23], colorectal [27]. The role of CAV1 in aging has been studied in the sense of response to growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) [24,25]. An elevated expression of CAV1 has been associated with senescent cells and the responsiveness of these cells for growth factors and re-enter cell cycle has been achieved with lowering caveolin levels [24]. Here we relate no differences of methylation status between young and aged individuals. However, we may speculate that the related change in expression of *CAV1* described by Cho et al. [24] may not been regulated by DNA methylation, but it can regulated by different epigenetic modifications such as histone methylation or acetylation or microRNA control.

When taken together the DNA methylation analysis of several genes studied by our group in two moments, in the same sample, we confirmed specific promoter methylation increased in older subjects, as reported previously by others [28,29]. Although our sample size is small, our result corroborates a genome-wide DNA methylation in a large cohort finding that 80% of age-related CpGs at island sites were increasingly-methylated with age [30].

The newly described age-associated methylated CpG sites contribute to recent data in the literature, trying to identify them, to better comprehension of the role of epigenetic variation in the aging process. Moreover, the differential methylation status in these age-related genes might affect the mRNA and protein expression in peripheral tissue of older individuals.

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