Vascular Smooth Muscle Polyploidization as a Biomarker for Aging and Its Impact on Differential Gene Expression

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Polyploidy is characterized by a greater than diploid content of DNA in a cell. Previous measurements of ploidy level in different organs of humans and rodents, including the aorta, indicated an increase in old versus young. We hypothesized that aortic vascular smooth muscle polyploidy is a biomarker for aging and that the augmented DNA dosage affects selective gene-specific transcript expression. Our results demonstrate that tetraploidy increases exponentially over the life span of the animal, serving as an indicator of age. Approximately 60% of the vascular smooth muscle cells in the thoracic aorta of 36-month-old Brown Norway rats are tetraploid compared with 5% in their 3-month-old counterparts. Microarray analysis and reverse transcriptase-PCR was performed with mRNA isolated from sorted diploid (2N) and tetraploid (4N) vascular smooth muscle cells from old rats to identify differentially expressed transcripts. For the majority of detectable transcripts, an increase in DNA content led to a proportional increase in mRNA. A select group of transcripts, however, were reduced in tetraploid compared with diploid cells. These mRNAs correspond to guanine deaminase, to the matrix proteins rat glypican 3 (OCI-5) and decorin, as well as to the inflammation-associated transcripts, insulin-like growth factor-binding protein 6, macrophage inflammatory protein 2 precursor, macrophage galactose N-acetylgalactoseamine-specific lectin, and complement component C4. Our study is the first to describe aortic polyploid level as a biomarker for aging and to indicate that changes associated with increased DNA content per cell may selectively suppress the expression of specific genes.

Polyploidy, which is the state of having a greater than diploid content of DNA, has been observed in a variety of mammalian cell types including hepatocytes (1), arterial smooth muscle cells (2), megakaryocytes (3), cardiac myocytes (4), and Purkinje cells (5). Furthermore, polyploid cells may be found in certain tissues under conditions of stress, such as uterine smooth muscle cells during pregnancy, thyroid cells during hyperthyroidism, and in the seminal vesicles with aging (3). Cellular polyploidization also occurs in cancer but this genomic instability more often leads to the development of intermediate DNA ploidy values, which are indicative of aneuploidization (6).

Arterial vessels thicken during aging and this process is mediated by an increase in smooth muscle cell mass and deposition of connective tissue. The increase in vascular smooth muscle cell (VSMC) mass is primarily due to the hypertrophy of preexisting cells, which occurs concomitantly with polyploidization, and not hyperplasia. No information is available, however, on the degree of polyploidization over the lifespan. Hyperplasia, which is characterized by an increase in VSMC proliferation, occurs during atherosclerotic plaque development (7) and balloon injury (8). Although chromosomal alterations in VSMC have been demonstrated to occur in atherosclerotic plaques (9), the plaques themselves were found to contain less tetraploid nuclei than the corresponding media (10).

Eukaryotic cells are generally prevented from becoming polyploid, due in part to the mitotic spindle checkpoint, and do not exit from mitosis until the mitotic cyclin, cyclin B, is successfully degraded. Recent studies of cultured VSMC have demonstrated that perturbations of the mitotic cell cycle checkpoint by overexpression of Akt/PKB (11), a serine/threonine kinase that mediates signaling via the insulin-like growth factor and platelet-derived growth factor receptors, leads to polyploidy. Additionally, overexpression of Cks 1, a Cdc2 kinase adaptor protein that promotes the degradation of cyclin B, also results in the development of polyploidy (12). It was suggested, but not proven by structural examination, that a premature exit from M-phase and cell cycle re-entry is responsible for achieving high ploidy in this cell type.

Currently, there are only two studies that have examined differential transcript expression as a function of ploidy. One investigation examined isogenic yeast that differed only in ploidy and the other used cDNA-amplified fragment length polymorphism to examine transcriptional differences in isogenic diploid versus autotetraploid Arabidopsis thaliana plants (13, 14). In both investigations it was concluded that a selected group of genes was not expressed proportionally to DNA content. In the current study, we have refined a cell sorting method to isolate large quantities of aortic VSMC that are fractionated based on their DNA content and have shown that polyploidy-dependent mechanisms of gene expression occur within a mammalian system. There are only a few markers that serve as accurate predictors of age. Our study also concluded that aortic VSMC ploidy level can serve as a biomarker for aging.

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Vascular Smooth Muscle Polyploidy

| Primer<sup>a</sup> | Sequence (5'-3') | Position and length<sup>b</sup> | Anneal. temp.<sup>c</sup> | Reference sequence accession no. |
|-------------------|------------------|-------------------------------|----------------|---------------------------------|
| CD94 for          | CTTGGAAATGGGATTGTCGCC | 443–772                       | 58  | NM_017245                      |
| CD94 rev          | AGGGCGGATGGGATTGTCGCC | 351                           | 62  | U42719                         |
| COMP. C4 for      | GAGACGAGGATTGTCGCC | 162                          | 52  | X98959                         |
| COMP. C4 rev      | AGGGCGGATGGGATTGTCGCC | 232                           | 52  | NM_031776                      |
| DGN for           | AGGGCGGATGGGATTGTCGCC | 735–1181                      | 52  | AF106860                       |
| DCN rev           | AGGGCGGATGGGATTGTCGCC | 1064–1633                     | 56–58| M96055                         |
| GDPH for          | TCCATCCATGAGTATGGTCGCC | 570                           | 58  | NM_022393                      |
| GDPH rev          | AGGGCGGATGGGATTGTCGCC | 1272–1420                     | 58  | NM_022393                      |
| G2A for           | CTGGTGCTGCTGCTGCTGCTGCTG | 170                           | 58  | M96055                         |
| G2A rev           | AGGGCGGATGGGATTGTCGCC | 550–1151                      | 58  | NM_022393                      |
| GML for           | TGGAAATGGGATTGTCGCC | 624                           | 60  | U54965                         |
| GML rev           | AGGGCGGATGGGATTGTCGCC | 62–280                        | 60  | M22400                         |
| MIP-2 for         | AGGGCGGATGGGATTGTCGCC | 219                           | 60  | M22400                         |
| MIP-2 rev         | AGGGCGGATGGGATTGTCGCC | 474–831                       | 60  | M22400                         |
| OCI-5 for         | AGGGCGGATGGGATTGTCGCC | 488                           | 60  | M22400                         |

<sup>a</sup> for, forward; rev, reverse.

<sup>b</sup> Position defined by the location on the reference sequence. PCR amplicon size is indicated in base pairs (bp).

<sup>c</sup> Annealing temperature.

EXPERIMENTAL PROCEDURES

Experimental Animals—Inbred Brown Norway rats were obtained from the NIA colony maintained by Harlan Sprague-Dawley (Indianapolis, IN) and were raised under specific pathogen-free conditions. All animals were used under an animal care protocol that was approved by the Boston University Institutional Animal Care and Use Committee. Male C57BL/6 mice at 3 months and 24 months of age were also obtained from Harlan Sprague-Dawley and inbred under specific pathogen-free conditions. Inbred Brown Norway rats were obtained from the Boston University Institutional Animal Care and Use Committee.

Flow Cytometric Analysis—VSMC were centrifuged and resuspended, at a concentration of about 2 × 10⁶ cells/ml, in DNA staining solution I (solution I: 50 μg/ml propidium iodide, 100 units/ml RNase A, 0.1% Triton X-100, and 4 mM sodium citrate) for 10 min at room temperature. DNA staining solution II (solution II: 50 μg/ml propidium iodide, 0.1% Triton X-100, and 400 mM sodium chloride) was subsequently added at a volume equal to that of solution I, and the solution was incubated in the dark at 4°C for at least 2 h prior to cytometric analysis. Flow cytometry was performed on a BD Biosciences FACScan. Laser excitation at 488 nm was produced by a 15-milliwatt argon-ion laser. Fluorescence collection for fluorescein isothiocyanate (green) and propidium iodide (red) was performed with a 530/30 (FL1) and 585/42 (FL2) bandpass filters, respectively. A minimum of 10⁶ events were analyzed per sample, and all experiments were repeated at least twice. The doublet discrimination parameter was employed on integrated and peak DNA signals to exclude cell aggregates. Statistics and graphics were calculated with the CELLQuest™ software (BD Biosciences).

Total RNA and Microarray Preparation—Total RNA was isolated from sorted cells with TRIzol Reagent (Invitrogen). RNA isolation was performed as per manufacturer’s instructions. For analyzing the integrity of total RNA, 2–5 μg was electrophoresed on a 1% agarose-formaldehyde Northern gel. RNA quantification was also performed with spectrophotometric analysis (1 OD = 20 μg of total RNA at 260 nm). The gel was analyzed for ethidium bromide staining and transferred onto a nylon membrane. The transferred RNA was further analyzed by methylene blue staining after UV cross-linking (5 min in 5% acetic acid solution, 5 min in 0.04% methylene blue solution, and washed for 5 min in water). Clontech Atlas rat 1.2 and 1.2 II membranes (catalog number 6854-1 and 7856-1; www.clontech.com) each contain 1176 spotted cDNA fragments in addition to control, housekeeping cDNA spots. 5 μg of total RNA was used to generate cDNA probes as per manufacturer’s instructions using [α-32P]dCTP (10 mCi/ml from PerkinElmer Life Sciences; number BLU-513H). The probe was purified as per the manufacturer’s instructions for the QIAquick Nucleotide Removal Kit (Qiagen, Valencia, CA). After purification, 200 μl of 10 mg/ml sheared salmon sperm DNA and 5 μl of rat C0t-1 DNA (1 mg/ml) were added to the probe, and the membranes were allowed to hybridize overnight at 67°C. The membranes were washed according to the manufacturer’s instructions and subsequently exposed to a storage phosphor screen for 5–5 days and evaluated with a PhosphorImager (Amersham Biosciences). AtlasImage for Windows software (Clontech) was used to quantitate transcript expression and for normalization. To determine differential transcript expression, each experiment was normalized based on global gene expression. For each gene represented on the arrays, the ratio of transcript expression was determined by dividing the adjusted signal intensity of the 4N cells by that of the 2N cells. Three separate batches of RNA were prepared for each array hybridization, using at least two different membranes. A mean ratio and S.D. (σ) were calculated for each gene-specific transcript expression.

RT-PCR Analysis—RT-PCR was conducted as follows: 5 μg of total RNA was diluted to 14 μl with dH2O, and 8 μl of oligo(dT)12-18 primer (0.5 μg/μl in dH2O) was added. The mixture was heated at 70°C for 10 min.
min and then cooled on ice. The following reagents were subsequently added: 12.4 μl of 5 × RT buffer, 2 μl of 0.1 mM dithiothreitol, 3 μl of dNTPs (dATP, dCTP, dGTP, and dTTP at 20 mM each), 3 μl of Super-script II RNase H-RT, and 20 μl of H2O. The mixture was incubated at 42 °C for 90–120 min. Cycle number was varied to ensure that amplicons were below the visual saturation. PCR primers and conditions are listed in Table I.

**Statistical Analysis**—The exponential function and R² value that were determined in Fig. 1B correlating ploidy level with the age of Brown Norway rats were calculated with Microsoft EXCEL (six data points). The criteria used for differential expression was defined by a ≥2-fold difference (18–22). This is a reasonable criteria because the p values of the genes that met this level are <0.05, a level associated with increased false negatives (23), and all differences were confirmed by RT-PCR with the exception of CD94. Microsoft EXCEL was used to calculate individual t tests (assuming homoscedasticity and type II) between individual transcript expression levels in addition to those of the control set of housekeeping genes (Table II).

**RESULTS**

**Aortic VSMC Polyploidization Increases Exponentially with Aging**—Age-dependent development of polyploidy was determined in both *Rattus norvegicus* and C57BL/6 mice. As shown in Fig. 1A, induction of tetraploidy in VSMC is significantly increased with aging in these two models. Both increases in ploidy profiles of 24-month-old animals compared with that of 3-month-old animals are statistically significant with a p value less than 0.001.

Given these results, we hypothesized that the degree of vascular polyploidization might serve as a biomarker for aging, namely, that this property is precisely programmed, allowing for the reliable prediction of age based on ploidy analysis. To examine this hypothesis, we resorted to the rat model for which different age groups were readily available. Fig. 1B exhibits the
Fig. 2. Analysis of sorted aortic VSMC. A, aortic VSMC derived from 24-month-old Brown Norway rats were sorted into 2N and 4N cell populations. Unsorted and sorted cells were re-analyzed by flow cytometry. Contaminating cells were quantitated and determined to be less than or equal to 10% of the total sorted population. B, sorted 2N and 4N cells were observed by phase contrast microscopy after sorting. Hoechst 33342 staining was visualized by ultraviolet illumination. Magnification was ×200. C, total RNA was isolated from sorted 2N and 4N cells and electrophoresed on an agarose gel. RNA was capillary transferred onto a nylon membrane which was subsequently stained with methylene blue. 28 S, 18 S, and 5 S ribosomal bands are indicated. Lanes 1, 2, and 3 are total RNA isolated from liquid nitrogen snap-frozen 3-month-old aortic tissue, 24-month-old aortic tissue, and brain homogenate, respectively.
percentage of tetraploid VSMC that were freshly dispersed from the BN rat aorta over the following six time points: 3 months (n = 5), 11 months (n = 3), 17 months (n = 3), 24 months (n = 22), 31 months (n = 3), and 36 months (n = 3). An exponential curve function was plotted to fit the six points, and an R² value of 0.996 was calculated. There is a similar increase in the percentage of 4N cells as well as in the ratio of 4N/2N (age ≥ 33 months). The R value (correlation coefficient squared or coefficient of determination) exhibits a very low total variation. Such correlations contain an inherent predictive value and would allow for the reliable prediction of the age of an animal based on aortic ploidy content. Indeed, a blind examination of aortic VSMC ploidy level enabled us to predict animal age with precision.

DNA-dependent VSMC Sorting—To examine the profile of gene-specific transcripts expressed in tetraploid VSMC compared with diploid cells, we adopted a cell sorting method for VSMC. We confirmed that the sorting procedure was successful by examining sorted cells with a separate nuclear staining technique and flow cytometry. A post-sort quality control analysis was conducted after each isolation and contaminating cells were consistently <10% of the total population of cells obtained (Fig. 2A). Hoechst 33342-stained, sorted cells (DNA stained) were subsequently analyzed by light microscopy and under ultraviolet illumination as exhibited in Fig. 2B. Visual examination further confirmed that the sorted VSMC were well dis-aggregated and fractionated into diploid and tetraploid cells. It was also confirmed that total RNA isolated from the sorted cells was intact (Fig. 2C).

Ploidy-dependent Differential Transcript Expression—In yeast and plants, an increase in ploidy induces differential expression of few selected genes (13, 14). RNA isolated from diploid and tetraploid aortic VSMC from 24-month-old Brown Norway rats was analyzed and subjected to microarray analysis of transcripts. Analysis of total RNA isolated from equal numbers of diploid and tetraploid cells indicated that the amount of RNA in the tetraploid cells was ~2-fold greater than that in the diploid cells. This was statistically significant, p < 0.05 (Fig. 3A). Similarly, a separate study demonstrated that tetraploid and octaploid VSMC exhibit a 2.4- and 4.8-fold increase in mass, respectively, compared with that of a diploid cell (24).

Previous investigations have demonstrated that limited proliferation occurred within the aorta during aging (24, 25). This suggested that the number of cells recruited to engage the cell cycle is small at any time point but gradually accumulates over a lifespan. We performed Western blot analysis for proliferating cell nuclear antigen, which is a polymerase δ accessory protein and a marker of S phase (26). As shown in Fig. 3B, proliferating cell nuclear antigen was not detectable in diploid or tetraploid cells, whereas it was prominently expressed in actively proliferating VSMC in culture. With this observation, we proceeded to explore differential cDNA expression in diploid versus tetraploid cells and related this to DNA content and not to the proliferative state of the cell.

The results of the microarray experiment, in which sorted cells were profiled, showed that, in general, the levels of RNA detected were proportional to the total RNA or DNA content in VSMC. Namely, in equal amounts of RNA analyzed in the diploid or tetraploid cells, the majority of transcripts displayed a relative expression ratio of approximately one (Fig. 4A), which includes those belonging to standard housekeeping genes (Table II). Furthermore, relative expression levels of each transcript were plotted (Fig. 4B) and indicate a very similar expression profile in the 2N and 4N cells. Out of 2352 genes surveyed, 322 gene-specific transcripts were found to be expressed, of which 7 mRNAs displayed a ≥2-fold decrease in expression in tetraploid versus diploid cells. Table III lists displayed transcripts in aortic VSMC by accession number in GenBank™, the mean expression ratio in tetraploid versus diploid cells, S.D., and associated p values. The criteria used for differential expression is defined by a ≥2-fold difference in groups analyzed (4N versus 2N), as also applied in other studies (18–22), and p values of <0.05, a level associated with increased false negatives (23). A list of all expressed transcripts is exhibited under Supplemental Material. The expression trend for each of the transcripts listed in Table III, except for CD94, was further confirmed by semiquantitative RT-PCR. It is possible that false positive results could be generated from cross-hybridization of one member of the C-type lectin family (CD94) (27). In addition to the list of cDNAs shown in Table III (selected based on the above criteria), we focused on cDNAs in which the p value was less than 0.05, such as decorin. Decorin was repeatedly confirmed by RT-PCR as being significantly reduced in 4N cells (based on Kodak Imaging). All amplicons were single bands and were titrated within the linear range of PCR amplification. Representative examples of OCI-5, guanine deaminase (GDA), and the control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are exhibited in Fig. 5. Hence, RT-PCR assays confirmed the general expression trend observed by microarray analysis, but the precise extent of reduction varied between the array and RT-PCR assays (as also reported in other studies, e.g. Ref. 28). Our study proved the contention that the state of ploidy selectively affects levels of mRNA transcripts in VSMC.

**DISCUSSION**

In selecting an animal model for aging, we have been guided by several considerations (reviewed in Ref. 29). Three inbred species of rat are currently available at the NIA: Fisher 344 (F344), Brown Norway (BN), and the F344BN hybrid. Due to
**TABLE II**

Relative expression ratios of housekeeping transcripts detected by microarray analysis are shown with GenBank™ accession numbers and the relative expression ratio of 4N versus 2N cells. An average of this ratio was calculated for three independent RNA preparations, and each was screened with at least two separate microarray filters.

| Gene                                | GenBank™ accession no. | Mean ratio | S.D. (σ) |
|-------------------------------------|------------------------|------------|----------|
| 40 S ribosomal protein S29          | X59051                 | 1.12       | 0.21     |
| β-Actin                             | V01217                 | 1.05       | 0.09     |
| Glyceraldehyde-3-phosphate dehydrogenase | M17701               | 1.11       | 0.31     |
| Hypoxanthine-guanine phosphoribosyltransferase | M63983             | 0.89       | 0.16     |
| Polyubiquitin                       | D16554                 | 1.05       | 0.15     |
| Tubulin α-1                         | V01227                 | 1.17       | 0.22     |

**FIG. 4.** Relative transcript expression levels in sorted VSMC. A, the relative expression levels of 322 gene-specific transcripts detected in diploid and tetraploid VSMC were ordinated from the lowest level to the highest. The S.D. of each relative transcript level is displayed as ± y axis error bars and is representative of three separate experiments, each of which comprised a total of 16 Brown Norway rats. B, relative expression levels of all transcripts detected in 4N and 2N vascular smooth muscle cells. Levels of expression are exhibited in arbitrary units on both axes.
Table III
Differentially expressed transcripts in sorted 2N and 4N aortic VSMC

| Gene          | GenBank™ accession no. | Mean ratio | S.D. (σ) | p value | Confirmation by RT-PCR |
|---------------|------------------------|------------|----------|---------|------------------------|
| GDA           | AF026472               | 0.05       | 0.05     | 0.01    | +                      |
| IGFBP-6       | M69055                 | 0.27       | 0.03     | 0.00    | +                      |
| MIP-2         | U54965                 | 0.38       | 0.08     | 0.01    | +                      |
| MGL           | J05495                 | 0.47       | 0.07     | 0.03    | +                      |
| Complement C4 | U42719                 | 0.50       | 0.10     | 0.04    | +                      |
| CD94          | AF099133               | 0.50       | 0.11     | 0.02    | +                      |
| OCI-5         | M22400                 | 0.51       | 0.16     | 0.01    | +                      |

FIG. 5. RT-PCR confirmation of relative transcript level. Semi-quantitative RT-PCR was used to confirm differentially expressed transcripts in sorted 2N and 4N VSMC. Representative examples of developmentally regulated intestinal protein (OCI-5), also referred to as glypican-3, and guanine deaminase (GDA) at two PCR cycle times are exhibited to confirm the down-regulation of this transcript in tetraploid compared with diploid cells. Three independent experiments, each with a separate RNA preparation, yielded similar results. GAPDH, glycer-aldehyde-3-phosphate dehydrogenase.

limited literature involving studies of VSMC with F344BN hybrids, we limited our final decision between the F344 and BN species. The Fisher 344 strain has several potential problems including a relatively short life expectancy and high prevalence of kidney disease, unless fed with a soy diet (30). The BN rat has been demonstrated to produce reduced plasma levels of kininogens and to have lowered kallikrein-like activity (31). The kallikrein-kinogen-kinin system is associated with blood pressure via the vasodepressive activity of endogenous kinins. We decided to study the Brown Norway strain also because previous investigations have shown that these rats have increased neointimal hyperplasia in response to vascular injury (32) and are susceptible to spontaneous rupture of the internal elastic lamina (33). Vascular insult resulting from physiologic stresses such as hypertension and aging is associated with increased polyploidy. Our study is the first to demonstrate an exponential relationship between the frequency of tetraploid aortic VSMC and age. Past studies have reported a significant increase in the frequency of tetraploid VSMC for both the Wistar Kyoto (normotensive) and Spontaneously Hypertensive Rat (hypertensive) models below 5 months of age (24). Increases in the population of tetraploid aortic cells as a function of age in humans have been reported as well (10). Our results with an aging model demonstrate an exponential association with a correlation coefficient of $R^2 = 0.986$ for the appearance of tetraploid cells over the lifespan of Brown Norway rats, which indicates a reliable predictive value. In fact, we suggest that the age of these animals could be predicted by measuring the ploidy content of aortic VSMC. Interestingly, one study reported an age-related increase in rat liver polyploidy that reached a plateau at around 80% tetraploid and 10% octaploid between 4 and 6 months of age (34). We also examined the generality of the phenomenon reported in our study by analyzing ploidy level in C57Bl/6 mice of different ages (Fig. 1). This strain was chosen also because it is frequently used in cardiovascular studies.

The mean age at which 50% mortality occurs in Brown Norway rats is reported to be 32 months, with a maximum lifespan of 40 months of age (35). We have shown that 36-month-old Brown Norway rat aortas contain $\sim60\%$ tetraploid VSMC. It is reasonable to suggest that this process is an integral part of advanced age because all of the surviving animals, without notable health issues, are those in whom this process occurs. A physiologic theory of aging in mammals suggests that the accumulation of DNA damage, due to reactive oxygen species and other genotoxic agents over time, is responsible for a decline in cellular function. DNA damage thus leads to genomic instability, and as a compensatory result, polyploidization may occur (36, 37). Similarly, others have suggested that in the case of hepatocytes, polyploidization protects the cell from dominant expression of mutated oncogenes (38). An additional conjecture is that polyploidy is a marker for a terminally differentiated state of an aged cell (39). These theories about the presence and development of post-mitotic tissue polyploidization are not mutually exclusive. Consistent with these contentions is the finding of increased fibroblast polyploidy in genetically modified mice, such as PARP-1 and p53, that are deficient in genes responsible for maintaining genomic integrity (40, 41). In general, these studies demonstrate that under experimentally induced conditions of genomic instability, polyploidization is a common marker of cells in a senescence-dependent cell cycle arrest (41).

In yeast, a total of 17 genes, out of $\sim6200$, exhibited ploidy-dependent expression that was independent of yeast mating type (14). Although the mechanism of this selective regulation is not clear, it was concluded that ploidy-regulated genes had an unbiased distribution of locations in the yeast genome. We show that in mammalian cells too, changes associated with an increase in DNA content per cell might be selectively suppressive of gene expression. Our microarray analysis of 2352 genes on sorted 2N and 4N VSMC indicated that a select group of transcripts among 322 expressing ones was down-regulated in the 4N cells. A different study employing a similar microarray
analysis in human aortic VSMC reported 145 gene-specific transcripts expressed out of 1176 surveyed (22), in the range detected in our investigations. The transcripts down-regulated in tetraploid VSMC include macrophage inflammatory protein 2 (MIP-2) (42), complement component C4 (43), and Gal/GalNAc-specific lectin (MGL) (44), all of which are involved with inflammation. The growth factor, insulin-like growth factor-binding protein 6 was also found to be down-regulated in tetraploid cells. Similarly, the matrix molecule rat glicyan was expressed at lower levels in tetraploid VSMC. In the search for potential common regulators of the displayed genes, we noted that they are not localized on any one or selective few chromosomes. Interestingly, however, these transcripts have been reported to be regulated by methylation or to contain several putative methylation sites (45–47). It is possible that with a wider search of genes, a few might be revealed as up-regulated in the tetraploid VSMC.

In summary, this is the first report of differential gene expression occurring in a DNA dosage-dependent mechanism in a mammalian system, and to point to VSMC ploidy level as a biomarker for aging. Future analyses should reveal whether the ploidy-suppressed transcripts are similarly reduced in tetraploid cells of other lineages in old animals and explore the functional relevance of each of these displayed genes.

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