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Review Article

Ageing Markers in Primary Fibroblasts of Healthy Middle Aged Persons and their Psychological Correlates: Review and Four Case Studies

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Abstract

The paper presents preliminary results of a novel methodology of systematic analysis of ageing markers by healthy middle-age volunteers, and their possible psychological correlates. Ageing markers detected in diploid fibroblasts were divided into three groups representing different types of normal ageing. Psychological processes possibly linked to these groups, especially primitive types of psychological defense strategies, intrinsic religious attitudes, and levels of psychological activation, are discussed. The paper is centered upon four case studies, accompanied by selected experimental data, and preceded by literature review.

Concept of Aging Markers

Primary non-transformed cell cultures tend to change with each passage: population doubling time increases, cell morphology changes. Differences between cells gradually increase, leading to the appearance of different subpopulations, primarily the subpopulation of old cells. The latter are larger; less elongated, and often rounded ones. Gradually, number of larger cells grows, and cell division in the population practically stops [1,2].

Simultaneously biochemical markers appear, regarded currently as aging markers, proper for aging cells in culture. Primary skin fibroblasts from patients of different age, as well as from patients with progerias (Hutchinson-Guilford and Werner syndromes) or, syndromes with signs of accelerated aging, also called "segmental progerias" (ataxia-telangiectasia, Sekkel syndrome, Cockayne syndrome), normally serve as models for the identification of aging markers [3]. In fibroblasts from progeria patients, a much larger number of aging markers has been detected than in fibroblasts from young healthy donors [4]. Aggregation of aging markers at the cellular level is currently regarded as predictive of chromatin changes, nuclear and cytoplasmic skeleton, level of unrepaired DNA damage, etc.

The first one of the suggested aging markers was lysosomal senescence-associated β-galactosidase (SA-β-gal), whose activity is dramatically increased in cells from old donors and ageing primary cell lines [5,6]. This marker has not lost its value and is currently actively used in oncology. The increased level of SA-β-gal in the biopsy material can serve as a serious basis for predicting the course of the disease. For example, pituitary adenoma shows that the presence of SA-b-gal and other aging markers explains tumors of this type being predominantly non-invasive, and not having metastases [7].

The second important marker of cellular aging is γ-H2AX. To date, we can characterize the role of γH2AX in the cell: persistent focuses of γH2AX are, firstly, a common phenomenon in the nuclei of human and animal cells after DNA damage and, secondly, they mainly accumulate in telomeric DNA and aging cells. They serve as a reliable cell marker for accelerated aging syndromes. Finally, the preservation of these foci for a long time after the action of ionizing radiation can serve as a marker for biological dosimetry, while their dynamics after the anticancer chemo- and radiotherapy can be used to assess their individual effect [8-12].

New proteins are being constantly detected and described, whose presence is necessary for the phosphorylation of γH2AX in response to DNA damage. TRAIP may serve as a plausible example of such protein, being one of the E3 RING ubiquitin-ligases. Mutations in the corresponding gene lead to disruption in the regulation of the cell cycle and, as a consequence, microcephaly and dwarfism [13]. TRAIP can bind to PCNA in a conservative site at the C-terminus in the region of stopped replication forks and is an active participant in the cell's response to replicative stress after DNA

damage. Absence of this protein makes it impossible to restore the movement of the replication fork, which leads to the chromosome instability [14]. Detection of γH2AX is actively used in present-day practical and experimental oncology [15,16].

Anti-oncogene P53 protein is now considered to be one of the aging markers. A number of functions and activities have been traced back in its case, namely, transcription regulation [16], involvement into DNA damage recognition and subsequent repair process [17]. In normal fibroblasts, p53 is usually not detected by indirect immunofluorescence method, as it grows stabilized and becomes visible in cells with damaged DNA or, in the aging ones.

P21, which acts as inhibitor of cyclin-dependent kinases, under direct p53 transcription control [18], is also an aging marker.Another traditional marker of repair processes is the protein 53BP1 - p53-binding protein 1 [19], often colocalized with γ-H2AX [20].

The main protein kinases activated in response to DNA damage are those belonging to the family of phosphotidyl-inositol-like kinases ATM, ATR and DNA-PK, as well as their targets, i.e. Chk1 and Chk2 kinases. Their appearance in the cell is also associated with aging processes, therefore they are also currently considered as markers of aging, and of DNA damage, and are used in applied research [15]. Conjunction of these markers with γH2AX shows the difference between the old and young cells in their response to bleomycin [21].

Increased amounts of gamma-H2AX and p-Chk2 serve as clear indication of fibroblasts from patients with Down's syndrome, often described as segmental progeria. It tends to correspond to increased amount of oxidative damages, such as 8-oxodG and enzymes involved in base excision repair - XRCC1 and DNA-polymerase β [22]. In this way, the pattern of aging markers associated with violations of DNA repair processes is revealed, accompanied by the accumulation of unrepaired lesions, and the activity of the corresponding signaling pathways. Their joint activation in cells that are defective in repair serves as a plausible proof of their mutual connection. For example, fibroblasts of mice knockout by the polymerase η gene, responsible for the development of variant form of Xeroderma pigmentosum in humans testify to the presence of an increased level of γH2AX foci, along with a number of other aging markers, namely, p53, p16 (Ink4a), p21, SA-β-gal [23].

The activity of DNA repair processes tends to decrease with age. At the same time, the number of cells with unrepaired DNA damage increases; the number of cells experiencing apoptosis in response to damage, tends to increase too. This leads to a decrease in number of not only normal, actively proliferating cells, but also to a drop in the total number of cells in the body, including those that usually do not divide. Since the decrease in cellularity is one of the obvious signs of aging, the study of the activity of DNA repair and of the level of apoptosis serve as important indicators of the aging process.

Along with the age of the cells, their epigenetic status also changes. Epigenetic determinants include inherited changes in genes regulation, without changes in the DNA sequence itself. In the course of the recent years, the role of epigenetic mechanisms in the processes of carcinogenesis has been revealed [24], as well as in cellular and organismic aging [25-27].

The level of DNA methylation in aging cells has been proved to reduce significantly. Passive demethylation of heterochromatin, as well as the loss of efficacy of the DNA methyltransferase DNMT1, the main methyltransferase for DNA supporting methylation, may serve as the possible triggers of this process [28,29]. Expression of DNA methylase *de novo* DNMT3b, may serve as response to the reduction of supporting methylation. In aging cells, it hypermethylates CpG islands in the promoter of such tumor suppressor genes, as LOX lysyloxidase gene, p16INK4a, transcription factor RUNX3, etc. [30]. Hypermethylation of promoter CpG-islands of 'progeroid' genes, such the *LMNA* gene mutant in the Hutchinson-Guilford syndrome [31], and the WRN gene, the mutation in which is responsible for the manifestations of Werner's syndrome [32], described for some types of tumors, leads to suppression of their activity.

Post-translational modifications of H2A, H2B, H3 and H4 histones, the so-called "histone code", tend to play significant role in the functioning, including transcription regulation, and maintenance, of genome stability [33-35]. Definite modifications, such as methylation of histone H3 and H4 over residues of arginine (R) or lysine (K), are stable, and can underlie epigenetic control and maintenance of highly organized chromatin structures during cellular generations. Methylation of histone lysines H3 is possible at the 4, 9, 27, 36 and 79 positions. Two of these modifications are quite interesting, e.g. trimethylated forms of histone H3 on lysine at the 9th and 27th positions (Me3-H3-K9 and Me3-H3-K27).

The level of Me3-H3-K27 is quite high in tumor cells [29,36,37]. It has also been found that a decrease in the Me3-H3-K27 leads to decondensation of the inactivated X chromosome in females [38]. Accelerated telomere shortening in patients with Hutchinson-Guilford syndrome may also be associated with it [38]. It has been demonstrated that the amount of Me3-H3-K9 and Me3-H3-K27 decreases with age or, under accelerated aging, so it can serve as a sufficiently reliable marker of aging cells [4].

The number of methylated forms of histones is regulated by the activity of histone acetylases - by sirtuin proteins, in particular. The decrease in the activity of SIRT1, SIRT2 and SIRT6 is observed in cell aging. SIRT1 and SIRT2 are markers of aging. They can be used as indicator proteins in the course of detecting the effect of geroprotector antioxidants, together with others, e.g. SA-β-gal activity, γ-H2AX, p53, p21CIP1, p16INK4A [39].

Another important component of the regulation of heterochromatin is the *HP1* protein. Its level of expression and localization can also be considered as an epigenetic marker [40]. In mammals, the overexpression of *HP1α* and *HP1β* (but not *HP1*γ), leads to telomere fusion, and to their shortening by decreasing the interaction with hTERT, the catalytic telomerase subunit [41-43]. It was shown that the heterochromatin binding site for chromo-domain of *HP1* provides Me3-H3-K27 [44]. The amount of *HP1*γ can serve as a reliable marker of cell aging, sharply decreasing in cells of elderly donors and patients with progerias [4].

The phosphorylated form of *HP1*γ is involved as platform for the formation of heterochromatin foci - SANF (Senescence-Associated Heterochromatin Foci) [45]. It is assumed that it is the mechanism of formation of SAHF that determines the irreversibility of arrest of the cell cycle in aging. Thus it may be argued that epigenetic markers tend to form their own pattern of aging markers, independent of DNA repair processes.

On a separate basis, changes in the nuclear lamina are possible to be described. They have been carefully described in the case of the cells of elderly donors, and of patients with Hutchinson-Guilford syndrome, resulting from the accumulation of the aberrant product of the *LMNA* gene, progerin [4]. Results obtained especially by Skaffidi and Misteli, allow us to consider the structural changes of a nuclear lamina as the basic process for inducing aging markers, associated with different levels of cellular regulation. The nuclear lamina participates in a very wide range of cellular processes, i.e. the regulation of chromatin organization and the position of nuclear pore complexes, DNA replication, stabilization of telomere complexes, regulation [46,47], integration with the cytoskeleton [48] and apoptosis [49].

The phosphorylation of lamin A or non-farnesylated progerin was associated to the formation of spherical intranuclear lamin A droplets that accumulate protein kinases of the CDK family capable of phosphorylating lamin A at serine 22. There are probably progeria types in which the development of the disease is associated with disturbance of the phosphorylation of lamin A in serine 22. Clusters of non-farnelized lamin A in the karyoplasm were observed, not only in the lamina region [50].

To date, detailed study of mutations in the *LMNA* gene (encoding A-type Lamins), or the protease *ZMPSTE24* gene (encoding an enzyme involved in Prelamin A maturation, leading to accumulation of wild type farnesylated Prelamin A) leading to the formation of progerin - farnesylated form of lamin A - has made it possible to offer personalized therapies using antisense oligonucleotides, which block certain mutations [51]. Unfortunately, new severe forms of progeria of children (mandibulo-dysplasia) have been found, caused by c.163G>A, p.E55K and c.164A>G p.E55G missens mutations, where processing of prelamine A passes without accumulation of progerin [52].

Telomeres are the terminal parts of the chromosomes. The role of telomeres in aging processes has been analyzed in detail by V.Mikhelson [53]. Most studies have shown that telomere length depends on age and sex [54-56]. It has been proved that the length of telomeres in blood lymphocytes of centenarians is shorter than in younger age groups; it tends to be larger in women than in men [57]. Nevertheless, telomere lengths tend to be spread in a fairly wide range for different individuals of a particular age.

So far no clear correlation has been found between telomere length and tissue renewal time *in vivo,* and it is believed that telomere length is more of an individual characteristic [58,59]. At the same time, the possibility of influencing the process of telomere shortening, not only by medical methods, but also with the help of psychological techniques [60] seems to be extremely important for understanding the possibility of influencing aging processes at the cellular and organism level. Probably changes in the nuclear lamina and the shortening of telomeres may be considered as another set of patterns of aging markers. Of course, these three tentatively detected groups of aging markers are related to each other - and most often appear themselves together. A number of highly constructive associations may be cited in this respect. Thus mutations in the gene of lamina A, leading to the accumulation of progerin, are often accompanied by decrease in the activity of the helicalase-nuclease WRN, which is traditionally associated with the processes of DNA repair [61,62].

Studies of recent decades have shown that cellular aging should be considered as a complex of heterogeneous but interrelated mechanisms in which products of genes defective in progeria, such as WRN, *LMNA* and ATM, are being involved in a direct or, indirect way. These mechanisms include disfunction of telomere complexes, and telomere shortening. Also, with cellular aging, a decrease in the efficiency of DNA repair is associated with the accumulation of various types of damage. Chromatin-specific changes in cell aging that affect the status of transcription, and the effectiveness of DNA repair, have also been described.

In the course of ageing, cells accumulate, whose nuclei have not the usual rounded shape, but are characterized by invaginations and bubble-like structures of the nuclear membrane [4,62]. The number of such cells can serve as an indicator of the number of "old" cells in the population.

Studying the dynamics of the formation of all these markers, we see that most of the aging markers seem to be related, since they reflect different aspects of the same process and should be described together. The appearance of a large number of cells carrying simultaneously several aging markers may serve as a sign of the formation of a population of senescent cells, the number of which ultimately triggers the aging process in a specific tissue and, more broadly, at the level of the organism.

Case studies

Materials and methods

Three volunteer subjects, 32, 46 and 47 years old, underwent medical examination and psychological testing. The former included taking skin biopsies, and obtaining primary fibroblast lines. Lines of primary fibroblasts from two healthy donors of 9 and 90 years, traditionally used by us as control, were taken for means of comparison in further analysis of aging markers. The preparation of cell lines, their management, antibody staining and data processing were performed according to the traditional scheme described earlier [63,64].

For means of determination of the actual of biological age of our respondents, a special methodology elaborated by V.Voitenko, was applied [65-71]. The methodology included assessment of a number of physiological parameters (body weight in kilograms, systolic blood pressure, pulse pressure (the difference between systolic blood pressure and diastolic pressure, duration in the delay in breathing after a deep inhalation, time of static balancing (on one foot), as well as a self-assessment questionnaire consisting of 28 entries.

The psychological part of our study consisted in six questionnaires which may be divided into three blocks: psychological tension (a), psychological defenses (b), and hidden reserves (3). Block (a) comprised two questionnaires, one of which consisted of 20 items, providing assessment of levels of psychological activation, tension, comfort, interest, and emotional activation [72]. Another questionnaire consisted of 45 items and measured the level of neuroticization [73]. The former of these inventories measured predominantly reactive (short-term) aspects of the psychological state of the subjects; the latter one measured its personal (long-term) aspects. Being combined, they became complementary, providing us with a reasonably accurate assessment of the psychological state of the

Ss, both in its short-term and long-term aspects. Both questionnaires were conducted in Russian, as all of our study, as Russian was the native tongue of all of our respondents. Both belong to the set of standard instruments of psychological investigation and diagnostics, applied currently in this country.

Block (b) comprised two questionnaires, one of which consisted of 50 items, providing assessment of level of activation of each of the eight major coping strategies, i.e. confrontation, estrangement, self-control, searching for social support, assuming responsibility, avoidance, planning of problem resolving, positive reassessment [74]. Another questionnaire consisted of 97 items, and measured the level of activation of each of the eight basic strategies of psychological defense, i.e. negation, suppression, regression, compensation, projection, substitution, intellectualization, and reactive formation [75,76]. The former of these inventories measured mostly conscious mechanisms of psychological defense, formed in the course of one's life; the latter ones measured the mechanisms which were mostly subconscious, and had been formed in the course of early ontogenesis. Being combined in our study, they could be regarded as complementary, providing us with a reasonable assessment of different aspects of psychological defense mechanisms, both conscious and subconscious.

Both questionnaires of block (b) belonged to the standard set of psychological inventories, applied in present-day Russian science. However both were based upon serious approaches, elaborated in the Western psychological tradition. In the case of the coping strategies, it was the transactional model of adaptation to stress constructed by R.S. Lazarus, with some valuable additions concerning the guidelines of coping behavior, by S. Folkman [77], in the case of basic defense mechanisms, it was the psychoevolutionary theory by R.Plutchik [78], complemented by some bright intuitions from the structural theory of personality by H. Kellermann [79].

Block (c) comprised two questionnaires, one of which consisted of 15 items, providing assessment of the level of intrinsic religiosity (that is, inner religiosity, as opposed to the extrinsic, outer one). This part of our study was based upon a well-known questionnaire elaborated by a prominent American psychologist of religion, Professor J. Kass [80]. It had been earlier adapted by us, in order to be applied in surveys of Russian speakers [81].

Another questionnaire consisted of 15 items, providing assessment of presence of features of short-term altered states of consciousness, often self-induced in stressful or unusual conditions in order to cope with psychological stress [82]. The 15 items were divided into five scales, giving assessment of presence of features of qualitative alterations of consciousness on the levels of perception patterns, emotional functioning, cognitive functioning, communicative patterns, and dream contents. This part of our study was based upon the concept of altered states of consciousness, elaborated by us as part of the scientific research program of the Human Brain Institute, Russian Academy of Sciences [83,84]. Questionnaires elaborated by R. van Quekelberghe and A. Dittrich, served as the nearest context of our inventory [85,86]. Including these two questionnaires into our research, we supposed that both of them were dealing with a vast realm, which could be tentatively called spirituality. In the case of extrinsic religiosity, we were dealing with its religious side (the so-called core religious experiences, to speak in strict terms of religious psychology); in the case of the altered states of consciousness, with its wider, non-religious one. Thus both inventories were regarded by us as complementary, i.e. presenting two different aspects of spirituality, which was regarded by us as a possible hidden reserve, recurred to in stressful or, unusual conditions in the service of the ego.

All of the psychological inventories applied in this study, belonged to the standard set of psychodiagnostic methodologies (with the obvious exception of the questionnaire of features of altered states of consciousness). It had been earlier successfully applied in our research of different psychological processes and states, by different groups of subjects [87-90]. All four respondents were normal middle-aged city dwellers, well-educated Russian speakers, being interested in the participation in our study for personal reasons.

Psychological profiles

Respondent A: was 22 years old, healthy by both his subjective feeling, and by objective parameters. He did not express any particular complaints concerning health. According to the psychological survey, he revealed low level of neuroticization, and fine psycho-emotional stability. His level of sincerity was high. At the same time, the respondent's attitudes were rather passive. The level of tension of the basic psychological defenses was moderate, among them negation was mostly active. This type of defense consists in ignoring unpleasant aspects of life, and denying its challenges, which in this particular case was well correlated with a generally optimistic view of him.

Respondent B: was 32 years old, healthy by both his subjective feeling, and by objective parameters. He did not express any particular complaints concerning health. The S revealed a serious attitude towards his health, for instance, he regularly underwent medical examinations. The level of neuroticism of the respondent B. was quite low. People of this kind tend to reveal emotional stability, communicative competence, ability to withstand challenges, and, as a result, high level of adaptation to stress. This indicator belonged to the so-called personal ones, which are normally formed early in the course of ontogeny, and not subject to changes in the course of everyday life. The insincerity level for this test was quite high (4, with the range of the corresponding interval from 0 to 5). This meant that respondent B. was able and willing to conform to social norms.

The general level of tension of basic psychological defenses was rather low in this case. Primitive, infantile defense mechanisms, normally formed early in the course of ontogeny, were fairly feeble here. As to the complex, mature ones, normally formed later in the course of ontogeny, they proved to be rather strong, especially the defense mechanism called intellectualization. The essence of this defense is formed by the S's ability to escape stress by means of rational interpretation of events, in order to find some kind of logic which would be subconsciously acceptable for the respondent. This indicator is also personal - that is, slightly changing in the course of life.

The levels of psychological activation, interest, emotional competence proved to be quite high, which was supported by the general will of the S to undergo a serious novel investigation. At the same time, some psychological problems were traced back. Thus self-esteemed level of psychological comfort was quite low, at the same time tension was really big. The causal relationship between these two indicators is subject to additional research (that is, it remains unclear whether the respondent felt uncomfortable in the situation

of medical inquiry and psychological testing, and compensated this feeling by means of increasing general psychological tension - or, on the contrary, he felt a strong tension for some reason, while lack of comfort was simply accompanying it). This index refers to the number of reactive ones, that is, those changing in the course of one's lifetime – and even in the course of one day.

Inner life of respondent B included some signs of transient (and, as a rule, short-term) altered states of consciousness, self-induced in the service of the ego. In structural terms, cognitive insights, as well as the communicative ones, tended to prevail. As to alterations of perception, as well as emotions, these were quite weak. This trend seems to correspond well to the prevalence of intellectualization in the structure of psychological defense mechanisms of this S. This cognitive pattern of dealing with stressful challenges tends to be more common by male persons in Russia, judging by our earlier studies. The opposite, emotional pattern tends to arise more often by female Ss. In general terms, this indicator is personal, that is capable of rather quick shifting.

The level of intrinsic religiosity proved to be rather high in this case. As the respondent B. pointed out in his response to one of the items of the Kass inventory (namely, item No.7), he feels inclined to review his life experiences, in order to search transcendental truth. Correspondingly, interest to spiritual and/or religious practices was in this case rather high. This attitude, including an intellectual effort, although not limited by it, corresponds well with protective intellectualization, proper for the given respondent.

We feel authorized to state in this case that spiritual insights, forming integral part of hidden reserves, tended to be in this case well-integrated into the general structure of psychological defense mechanisms, on the basis of intellectualization, which was traced back by us by means of three different inventories.

Respondent C: 46 years old, had some health problems (primarily, hypertension), but felt just fine in the course of our study. Application of our psychological inventory showed a sharply increased level of neuroticisation. Certain emotional instability often occurs in such cases, which may in its turn lead to irritability, anxiety or, general tension. In some cases, hypochondriac fixation on unpleasant somatic sensations is possible. The level of insincerity, measured by a special index, was minimal.

On the list of basic psychological defenses, regression served as the leading strategy. Regression is definitely a primitive one, formed in the course of early ontogeny. When a person grows adult, this gives in its turn rise to immature, infantile forms of behaviour, like stubbornness or, seeking comfort in the simplest possible way, like eating or, smoking.

The levels of psychological activation, of interest, of emotional tone, of inner comfort, of tension were close to normal in this case. The general psychological state of the respondent should be thus assessed as stable.

High level of presence of features of altered states of consciousness was found by us in this case, as well as the level of intrinsic religiosity. One could suppose that some peculiar traits of childhood could in this case condition definite behavioral passivity, compensated by rather active spiritual life.

Respondent D: was 47 years old, being quite healthy and active. The level of neuroticism was in this case quite low. Emotional stability and positive attitudes formed the core of this personality, providing firm basis for excellent stress adaptation. The level of insincerity is minimal.

The level of tension of basic psychological defenses was moderate. Negation served as the foremost defense. This strategy often implies denying and/or, ignoring the challenges of everyday life. This type of protective mechanisms is quite primitive, however often effective.

The levels of psychological activation, of interest, of emotional tone, of inner comfort, and of tension were close in this case to the corresponding norms. General psychological state was definitely stable.

The level of altered states of consciousness was close to average. The level of intrinsic religiosity was also quite moderate. The patient was thus rather far from application of spiritual and/or, religious means to withstand life stress.

Applying the Voitenko methodology, the difference between the biological age of each respondent, and his calendar one, was assessed. For results of this calculation as shown by these data, biological age was significantly than the calendar one in the case of respondents B. and D. Both assessments coincided by respondent C. In the case of respondent A, calendar age was lower than the biological one. These results lead us to the analysis of the genetic data (Table 1).

Ageing Markers and Discussion

Aging markers were registered and quantitatively evaluated in lines of primary fibroblasts obtained from the four respondents (Table 2).

As shown by Table 2, the number of aging markers in cells from the **respondent A.** does not practically differ from their number in the cells from a 9 year old healthy donor. This means that at the age of 22 years, the 'young' cellular phenotype dominates, not really being influenced by one's psychological characteristics and attitudes, including those having to do with one's health. As a consequence, the cells from both a young child and a young man can equally well serve in assessing the level of aging.

The number of aging markers from respondent B. was reduced insignificantly. In practical terms, we see here a picture which almost

Table 1: Comparison of the biological and the calendar age.

Respondent	Calendar age (full years)	Health self-assessment index	Estimated biological age (full years)	Due biological age (full years)	Degree of aging
A.	22	0	21	21	0
B.	32	0	26.8	30.2	-3.4
C.	46	5	52.8	47.5	+5.3
D.	47	6	31,1	44	-12.9

Table 2: Aging markers in diploid fibroblasts.

Age marker / Fibroblast line	Number of cells with γ-H2AX foci (%)	Number of cells with 53BP1 foci (%)	HP1-γ intensity of fluorescence (conventional units)	Number of cells with changes in nuclear lamina(%)	Telomere length (thousands of nucleotide pairs)
9 years 6 passages	1.2±0.9	6.3±0.3	170±12	1±0.7	9.24
9 years 25 passages	8.3±1.9	11.4±3.7	125±9	7±0.3	4.82
90 years 6 passages	12.2±2.7	18.3±3.3	85±16	10±1.3	3.71
22 years 6 passages (respondent A)	1.1±0.7	6.1±0.5	183±14	0.5±0.7	8.06
32 years 6 passages (respondent B)	0,8±0.2	4.2±1.1	168±11	0.7±0.3	7.69
46 years 6 passages (respondent C)	6,2±0.7	9.1±2.6	54±12	6±0.3	8.43
47 years 6 passages (respondent D)	3,2±0.4	7.2±1.4	133±15	8±1.3	3.24

coincides with the cases of respondents of both 9 and 22 years old. Thus it looks most plausible to suppose that caring for one's health and ensuring well-being forms in fact a quite effective strategy in order to retain a young phenotype. There is no doubt that there exist a lot of other factors that can influence one's biological age. However psychological attitudes are likely to play a significant role.

Fibroblasts of respondents C and D. were characterized by obvious accumulation of aging markers. Both belonged to roughly the same age (one was 46, another 47 years old), so some ageing markers, like the number of cells with 53BP1 foci, and the number of cells with alterations of a nuclear lamina did not reliably differ (both of them tend to increase with age). The difference lay in the amount of heterochromatin protein *HP1*-γ (which tends to decrease with age), and the number of γ-H2AX foci (which tends to increase with age). Respondent C. proved to be much older, judging by these two ageing markers, than respondent D [59].

These findings corroborate our hypothesis that at the age of roughly 40 to 45 years, a subpopulation of actively aging cells appears, which is not directly related either to biological age or, the presence of chronic diseases. One may also assume that this tendency, discovered by us on the level of chosen ageing markers, tends to increase and to become more visible in the course of further ageing.

At the same time, careful analysis proves that the situation is not as plain as it might seem. Thus the length of the telomeres from respondent C. was almost 2.5 times higher than that from respondent D, although the latter was somewhat younger and healthier. Telomere length is now considered to be one of important indicators of the general state of health. So we may assume that personal activity and creativity in handling one's psychological attitudes and behavioral patterns might affect at least some of ageing markers, especially, telomeres. This supposition is founded upon data acquired in different conditions, like hormone replacement therapy [66], therapy in patients with depression [67], and in stress management by Alzheimer's disease [68].

To sum up this line of research, respondent C. proved to be significantly different from the other three Ss: in psychological examination, only he demonstrated increased level of neuroticization, his biological age was more than 5 years longer than the calendar one (the other three Ss had biological age which was much lower than in the case of respondent C.), and he had more pronounced aging markers.

At the same time, such an important marker as telomere length was significantly higher in this case than the average age norm [59]. Taking into account results of psychological testing, we may remind that respondent C. demonstrated the prevalence of an 'infantile', regressive type of psychological defenses, accompanied by rather high levels of intrinsic religiosity, and of altered states of consciousness. We would like to point out here that a structurally similar hypothesis was formulated at a recent international conference by one of the leaders of ageing studies, V.Skulachev, in a report entitled Naked mole rats and humans: Highly social creatures prolonging youth by delay of ontogenesis (neoteny) [69]. Skulachev's main point here was that pertaining infantile features on different levels might greatly contribute to the given species' life expectancy.

In a much earlier report at another international conference, D.Spivak et.al. stressed the fact that the carriage of A2 allele of the type 2 serotonin receptor tends to correlate with intrinsic religiosity, on the one hand, and longevity, on the other hand [65,70]. Ability to self-induce short-term altered states of consciousness in the service of ego is currently supposed to function as a peculiar psychological defense mechanism, which may positively influence life expectancy.

Finally, the present study has corroborated our hypothesis that there exists in fact not a single group of well-correlated aging markers, but a number of these groups, at least three of them, which may be regarded as independent from each other:

1. **The first group** includes aging markers associated with unrepaired DNA damage - such as the γ-H2AX and 53BP1 examined by us in the present study, and also the phosphorylated form of the protein kinase ATM, the detectable levels of the P53 and P21 proteins, etc.;
2. **The second group** includes various epigenetic markers, such as the *HP1*-γ examined by us in the present study, as well as the trimethylated forms of histone H3 by lysine in the 9th and 27th positions; and the SIRT1, SIRT2, SIRT6 histone' deacetylases.

3. **The third group** is formed by such ageing markers as the severity of nuclear laminopathy, and the length of telomeres. As demonstrated in the present paper, respondent C., whose biological age exceeded significantly his calendar age, had sharply reduced amount of protein *HP1*-γ (belonging to the group of epigenetic markers), especially compared to respondent D., whose calendar age was practically the same. As to the differences between these two Ss in the number of markers associated with DNA damage, and of 53BP1, they tend to be insignificant. At the same time, telomere length by respondent C. exceeded that of the three other volunteers, being either much younger or, of the same age.

Conclusion

In order to obtain a well-founded assessment of the level of ageing, one has from the very beginning to regard not individual markers, however constructive, but a whole complex of these. One has primarily to take at least 1-2 markers from each of the three groups of ageing markers, proposed above, in the text of the present paper.

To cite a serious recent study, which has involved a number of Ss, large enough to allow full-scale statistical data processing, the number of aging markers is firmly related to biological age, which regularity tends to increase together with the age of the respondents [91,92]. Our data do not contradict with this finding. However we would assume that its general framework is somewhat more complicated, namely:

- at the initial stages of aging, different mechanisms of accumulation of aging markers are switched on. The same is true for different psychological protective mechanisms and reserves.

- at ageing process goes on, these differences first increase significantly, but later become less pronounced. The reason is that when a certain number of aging markers accumulate to a critical level, the cells cease to divide. As a result, they do not join the pool of those fibroblasts that can give rise to a primary cell line. Thus the older the fibroblast donor, the more likely that the number of aging markers in him/her would correspond to some near-threshold value.

Basing on these tentative conclusions, we find it most expedient and timely to create a database of ageing markers belonging to the three major groups proposed in the present paper, basing on data from a broad range of donors aged 30 to 70 years. Collecting a similar database of basic psychological defense mechanisms, coping strategies, and hidden reserves forms another urgent task. Comparing the two databases would be most constructive for the elaboration of a general concept of the molecular genetic markers of ageing, and their psychological correlates.

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