

Manifestations of ageing at the cytogenetic level

Alina WOJDA¹, Michał WITT^{1,2}

¹Institute of Human Genetics, Div. of Molecular and Clinical Genetics, Poznań, Poland

²International Institute of Molecular and Cell Biology, Warszawa, Poland

Abstract. The effects of ageing in humans appear to be a combination of influence of genetically programmed phenomena and exogenous environmental factors, and take place at the cellular level (senescence), rather than at the level of the organism. There are many processes, which occur in somatic cells as a consequence of DNA replication (accumulation of DNA errors or mutations that outstrip repair processes, telomere shortening, deregulation of apoptosis, etc.) and which drive replicative senescence in human cells. DNA errors are considered to be critical primary lesions in the formation of chromosomal aberrations. It can be concluded that the chromosome aberrations are biomarkers of ageing in human cells. Studies of human metaphases, interphase nuclei and micronuclei showed the increase in loss of chromosomes and the increase in frequency of stable chromosome aberrations as a function of age.

Key words: ageing, chromosomal aberrations, DNA damage, FISH, replicative senescence.

Introduction

The effects of ageing in individuals are determined by a cumulative effect of internal (genetic) and external factors that drive ageing (SEMSEI 2000). There are two main categories of ageing theories. Programme theories imply the existence an internal or external programme that determines the ageing process *ab ovo*. By contrast, error theories involve the idea that ageing would not happen without destructive factors that cause errors, mutations, deregulation of cellular processes, and in turn these processes finally lead to cellular dysfunctions and senescence. The universal mechanism would be consistent with both of above theories and it

Received: September 23, 2002. Accepted: February 20, 2003.

Correspondence: A. WOJDA, Institute of Human Genetics, Polish Academy of Sciences, ul. Strzeszyńska 32, 60-479 Poznań, Poland, e-mail: wojdal@man.poznan.pl

would assume that ageing is a sum of harmful effects in individual cells, rather than a process acting at the level of the organism (RAMSEY et al. 1995, GUARENTE 1996). There are several suggestions that ageing can take place at the cellular level:

I. Cellular ageing appears to be related to, and perhaps caused by, nuclear DNA and mitochondrial DNA damage and diminished DNA repair. Senescent cells share some molecular features: altered gene expression, disturbance of cell cycle duration, telomere shortening, genetic instability, deregulation of apoptosis (GUARENTE 1996). Therefore many of the processes that occur in most of somatic cells as a consequence of DNA replication (accumulation of DNA errors or mutations that outstrip repair processes, telomere shortening) drive replicative senescence in human cells (CAMPISI 1996).

II. Cell division occurs during life in many tissues either as a part of normal tissue function or in response to tissue damage. But after a limited number of cell divisions they reach a quiescent state termed senescence and become postmitotic (with few exceptions: spermatogonia, hematopoietic stem cells, tumour cells). The accumulation of cells at the end of their replicative lifespan in the elderly might contribute to tissue ageing (GUARENTE 1996, CATALAN 1995).

III. Dysfunction in many organs observed at an old age, is due to a loss or an altered function of cells. This includes the loss of neurons in the brain, loss of melanin production in hair follicle melanocytes, overexpression of collagenase and underexpression of collagenase inhibitors in senescent human skin (GUARENTE 1996).

The general process of cellular senescence is termed replicative senescence, in contrast to the process of ageing of the organism. Replicative senescence limits the proliferation of normal human cells, irreversibly arrests growth, and affects cell function. Replicative senescence is controlled by multiple dominant-acting genes (GUARENTE 1996).

Molecular features of senescent cells

Replicative senescence genes

Premature aging syndromes (Werner syndrome, Cockayne syndrome, ataxia telangiectasia, Blooms syndrome) originate from mutations in repair genes leading to impairment of DNA repair. The same process of aging can be caused by a replicative senescence gene. Till now many of these genes have been identified. A senescence-related gene *MORF - 4* (mortality factor on chromosome 4) was identified in the region 4q33 - q34.1. *MORF - 4* has a role in transcriptional regulation via chromatin remodelling by histone acetylation (BERTRAM et al. 1998, BRYCE et al. 1999, BERTRAM et al. 2001) The *PHLDA1* (pleckstrin homology-like domain, family A, member 1) gene has been mapped to 12q15. *PHLDA1* encodes a protein inducing cell death in T lymphocytes (KUSKE et al. 2000). Two replicative senescence genes are located in the subregion 6q21,

C6orf4 - 6 and *C6UAS* (KARAYIANNI et al. 1999, MORELLI et al. 2000). The *DNC11* gene in the long arm of chromosome 7 plays a specific role in cellular senescence, encoding a cytoplasmic dynein intermediate chain in senescent cells (HORIKAWA et al. 2001). Two senescence loci have been localized within 1q31 – qter and 1q42 – q43. These genes have a role in growth arrest (VOJTA et al. 1996). Some other genes may be located on 20q (CUTHILL et al. 1999). Several investigations have provided evidence for the localization of a telomerase suppressor gene, which induces cellular senescence on chromosomes 3 and 4 (BACKSCH et al. 2001).

DNA damage and repair

DNA repair can be defined in a general sense as a range of cellular responses associated with restoration of the genetic instructions, as provided by the normal primary DNA sequence (KIRSCH-VOLDERS et al. 2001). In cells of young and healthy individuals, spontaneous DNA damage and repair are in balance. The capacity to remove DNA lesions appears to diminish with age (WEIRICH-SCHWAIGER et al. 1994). Hence the lower level of DNA repair and/or the higher level of genomic instability are characteristic of old age. More than 70 human genes are directly involved in the major pathways of nuclear DNA repair. The expression levels of these repair genes fluctuate during the cell cycle under the control of cell-cycle-dependent transcription factors. On the other hand, high-level expression of some repair enzymes can delay growth in the G2 phase and stop the cells from progression into the S phase (KIRSCH-VOLDERS et al. 2001). These observations demonstrate the close relationship between DNA repair and cell cycle regulation. Consequently the deregulation of repair genes and repair enzymes can be associated with an acceleration of ageing (RONEN et al. 2001).

Recently various mutations and rearrangements in mitochondrial DNA and in nuclear genes encoding elements of the oxidative phosphorylation system have been described (RONEN et al. 2001). Mitochondrial DNA encodes 13 polypeptide components of the oxidative phosphorylation system. With the process of ageing, damaged mitochondria are unable to maintain the energy demands of the cell, leading to an increased production of free radicals. Therefore impaired ATP production or an increased production of reactive oxygen species resulting from mutations of mtDNA, reduce cell viability and may initiate cell death (FOSSLIEN 2001). In old individuals the ATP level in cells is lower, the rate of G2 repair is diminished and the G2-phase duration is longer. Thus mutations of mtDNA play a major role in the process of ageing (KROEMER et al. 2000, MULLER 2000, RONEN et al. 2001, WALLACE 2001, LUDWIG et al. 2001, BERDANIER et al. 2001, ZAHN et al. 2000).

Telomere shortening

Telomeres are specialized structures at the ends of eukaryotic chromosomes, composed of tandem repeats of a repetitive DNA sequence (TTAGGG)(n) and as-

sociated proteins. They have a number of important functions, including the protection of chromosomes from end-to-end fusion, degradation, elimination of genes during cell division and recombination. Successive shortening of the telomeres with each cell division (50-200bp) results finally in an arrest of cells in the G2/M phase, preceding DNA fragmentation and death of normal cells. Therefore telomere shortening and deficiencies in telomere repair are the best candidates for a cell division "counting" mechanism in normal somatic cells and play a key role in the process of genetic instability as well as cellular ageing (MULTANI et al. 2000, HAHN et al. 2001, CROWE et al. 2001, COTTLIAR et al. 2001, KLAPPER et al. 2001). Telomerase is the enzyme that maintains chromosomal length by synthesizing new telomeric DNA, preventing cell cycle arrest and inducing cellular senescence (CATALAN et al. 1995, MATTSON et al. 2001, SAMPER et al. 2001, BACKSCH et al. 2001).

Deregulation of apoptosis

Apoptosis, or programmed cell death, is a phenomenon that plays an important role in many physiological processes during the whole lifespan. It is involved in embryonal differentiation of organs and tissues. After birth and through adulthood, it helps to eliminate unneeded and damaged cells (CUTHILL et al. 1999, FENECH 2000, WENG et al. 2001). Apoptosis is often accompanied by growth arrest. However, cell cycle arrest does not always lead to cell death since the latter can be induced without the onset of cell cycle arrest (FENECH 2000, WENG et al. 2001). Many tumour suppressor genes and oncogenes are involved in the regulation of both the cell cycle and cell survival (DELORME et al. 2000, DECARY et al. 2000, WENG et al. 2001). In general, apoptosis occurs when the timing and order of cell cycle events are out of control or the damage of DNA is significant (WENG et al. 2001, CUTHILL et al. 1999). Several studies have shown age-related changes in the level of proteins (caspases) and factors that regulate apoptosis (REED 2001); these changes lead to deregulation of the process.

Chromosomal aberrations in senescent cells

In proliferating cells, most of the DNA damage capable to generate chromosomal aberrations is repaired during the G2 phase. It has been postulated that the G2 repair mechanism involves two cooperative pathways: (1) control of mitotic delay (G2 arrest) associated with DNA damage level and (2) DNA repair pathways for removing DNA lesions requiring ATP and NADH. Since chromosomal aberrations arise as a result of misrepaired or unrepaired lesions, the inhibition of G2 repairs should result in an increased number of chromosomal aberrations (PINCHEIRA et al. 1993). Repair-deficient cells may accumulate more DNA damage, resulting in an increased number of chromosomal aberrations with age (RAMSEY et al. 1995).

DNA breaks are considered to be critical primary lesions in the formation of chromosomal aberrations. They may be induced by exogenous agents but also occur spontaneously during cell cycle (PFEIFFER et al. 2000). Analysis of peripheral blood lymphocytes provides a possibility to assess structural and numerical chromosome damage *in vivo*. Lymphocytes are generated from hematopoietic stem cells in primary lymphoid organs, such as thymus and adult bone marrow. A portion of mature peripheral lymphocytes are long-lived and can persist in the blood circulation for several years in the quiescent non-proliferative G0 phase until they are activated by a specific antigen. Some of the cytogenetic changes observed in cultured and uncultured lymphocytes are considered to represent aberrations generated *in vivo*. The chromosomal aberrations in peripheral blood lymphocytes should be the best indicator in the detection of senescence. In "normal" somatic cells, the frequency of spontaneously occurring chromosomal aberrations is rather low, with incidence of 0.6% in human lymphocytes (CATALAN et al. 1995, SURRALLES et al. 1996, PRESSL et al. 1999). Skin fibroblasts can also be used to assess structural and numerical age-related chromosome damage *in vivo*.

Aneuploidy

Aneuploidy is defined as a deviation from the normal chromosome number of a cell or individual. Aneuploid cells with hypoploid or hyperploid chromosome numbers arise when chromosomes fail to segregate properly during mitosis. Several studies have shown a significant increase in chromosome loss (primarily of the sex chromosomes), seen in peripheral blood lymphocytes and skin fibroblasts in both males and females of advanced age. In some other studies, however, it was shown that the frequency of autosomal loss (hypoploidy) and autosomal gain (hyperploidy) is not necessarily a function of age or sex (NOWINSKY et al. 1990, HANDO et al. 1994).

Studies of metaphase plates

Sex chromosomes

Since the early 1960s, the rate of chromosome loss in human lymphocytes has been known to increase with advancing age. Studies of banded metaphases show that the loss most frequently involves the X chromosome in females and Y chromosome in males (CATALAN 2000, COTTLIAR 2001). Hypoploidy was more common in older individuals and was more evident in females because of an excess of 45,X cells. Sex chromosome gain was significantly more frequent in females than in males and was mainly attributed to 47,XXX. Sex-chromosome losses and gains were significantly more frequent in adult females than in adult males, but such differences were not evident between the younger male and female groups (NOWINSKI et al. 1990).

Autosomes

Studies of nonbanded metaphases confirmed an excess at 45-C cells in females and of 45-G cells in males, especially over the age of 45 years in females and 55 years in males. Hyperploidy also increased with age and in females was attributed to 47+C cells (for review see NOWINSKY et al. 1990). Loss of autosomes is negatively correlated with relative chromosome length (NEURATH et al. 1970, NICHOLS et al. 1978, SMITH et al. 1980, STOLLARD et al. 1981, BROWN et al. 1983, WENGER et al. 1984).

Studies of interphase nuclei

The above-described investigations are based on metaphase analyses. Although the analysis of metaphases permits the simultaneous assessment of numerical and structural aberrations, the technique has some disadvantages: it is time-consuming, allows the analysis of only those cells that are able to divide, and apparent hypodiploidy may be due to technical artefacts. This problem can be overcome by application of *in situ* hybridization (ISH) or fluorescence *in situ* hybridization (FISH) techniques with chromosome-specific DNA probes, enabling the direct analysis of large numbers of interphase nuclei. Therefore many authors applied these techniques to determine the frequency of aneuploidy as a function of age.

Sex chromosomes

The incidence of X-chromosome hypoploidy in females aged 1 week to 91 years is a common phenomenon. Even prepubertal females show X-chromosome loss, in women aged 0-15 years being about 1.5-2.5%, rising to 3.13% in women aged 16-50 years, and to 5.1% in women aged over 90. When the individuals were divided into two age groups on the basis of sex hormone function (pre- and postmenopausal individuals), a statistically significant correlation between X-chromosome loss and age was obtained clearly for women older than 51 years. HORSMAN et al. (1987) suggested that *in vivo* physiological variation of hormone concentration may play a role in X chromosome aneuploidy. Moreover, the oestrogen/progesterone balance of the donor at the time of blood collection may influence the rate of lymphocyte proliferation and mitotic behaviour (WHEELER et al. 1986). GUTTENBACH et al. (1995) suggested that sex-chromosome loss in females increases significantly only beyond the reproductive age (Figure 1).

The incidence of Y-chromosome hypoploidy in men is distinctly lower than that determined for X in women. The portion of Y-hypoploid nuclei is only 0.03% in children below the age of 5 years and remains relatively low until the age of 15 years (0.05%). In 16-20-year-old individuals the frequency rose to 0.24% and then continuously increased with age to a frequency of 1.34% in men 76-80 years old. The significant correlation between Y chromosome loss and age in males older than 16 years coincides with puberty and could be explained by hormonal changes, which in some way influence Y-chromosome loss. The in-

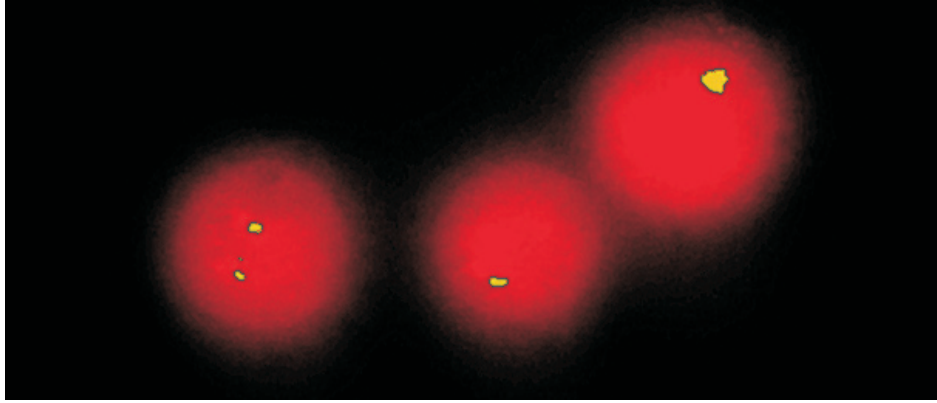


Figure 1. Loss of chromosome X. FISH with X α satellite chromosome probe in lymphocyte interphase nuclei from a female. Three interphase nuclei, one with two signals on each X chromosome and two with one signal.

creasing loss of the Y chromosome throughout adult life in males is reminiscent of the loss of the telomeric repeats that has been observed in human blood lymphocytes as a function of age. Some authors did not detect any correlation between the loss and donor age. This discrepancy might be explained by the non-consistent age distribution of studied individuals (GUTTENBACH et al. 1995).

Autosomes

MUKHERJEE et al. (1997) published the results of numerical chromosome analyses of skin fibroblasts at interphase and at metaphase. A study using the ISH method with chromosome-specific DNA probes of 17 different chromosomes, in-

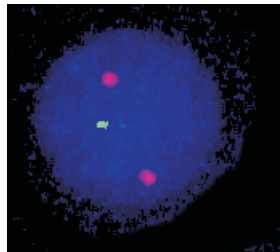


Figure 2. Loss of autosome. FISH with 1 (red) and 6 (green) α satellite chromosome probe in lymphocyte interphase nuclei. Interphase nuclei with two signals on each chromosome 1 and with one signal on chromosome 6. Loss of chromosome 6.

dicates that significantly higher percentages of aneuploid cells are detected at interphase than at metaphase. The levels of chromosome-specific aneuploidy increase with the donor's advancing age and, in most individuals, chromosomes 1,

4, 6, 8, 10, and 15 show significantly higher frequencies of aneuploidy at interphase than those of the other 11 chromosomes studied. Although the significance of relatively higher levels of aneuploidy of certain chromosomes occurring with age remains unclear, it is intriguing that some of these chromosomes, such as 1, 4, and 6, have already been confirmed to harbour senescence genes. Also chromosome 8 is known to house the gene for Werner syndrome (progeroid syndrome), characterised by limited proliferative capacity of fibroblasts. It is conceivable that the degree and/or type of chromosome-specific aneuploidy might have some gene dosage effects on the control of cellular proliferation and selection during aging. However, some authors question the correlation between autosome loss and age (GUTTENBACH et al. 1995) (Figure 2).

Studies of micronuclei

A dysfunctional kinetochore and centromere is likely to cause lagging of entire chromosomes or chromosomal fragments during anaphase. This phenomenon leads to a production of micronuclei during telophase. Micronuclei are produced from whole chromosomes or chromosome fragments that are left behind during nuclear division and appear in the cytoplasm of daughter cells as small additional nuclei (SURRELLES et al. 1996). DNA loss by the process of micronucleation is associated with ageing, cancer and environmental exposure (LEACH et al. 2001). Cells blocked with cytochalasin-B do not undergo cytokinesis. Application of *in situ* hybridization to cells blocked in cytokinesis by cytochalasin-B allows us to distinguish the pre-existing aneuploid nuclei from those arising *in vitro* (CATALAN et al. 2000, RICHARD et al. 1994, CATALAN et al. 1995, SURRELLES et al. 1996, HANDO et al. 1997)(Figure 3).

The association between aneuploidy and micronucleation was confirmed by the findings of FENECH and MORLEY (1985), showing that the age-related in-

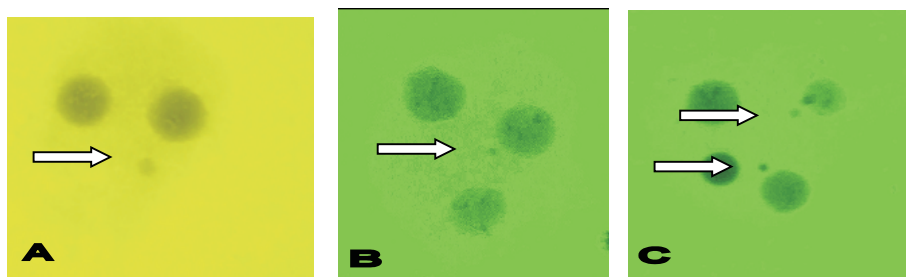


Figure 3. Presence of micronuclei in cytokinesis-blocked lymphocytes.

A = binucleated cell with one micronucleus, B = tetranucleated cell with one micronucleus, C = tetranucleated cell with two micronuclei. Arrowheads indicate micronuclei.

crease in frequency of micronuclei preferentially concerns kinetochore-positive micronuclei. The investigations based on the identification of kinetochores by antikinetochore antibodies have suggested that 34-84% of spontaneously occurring human lymphocyte micronuclei harbour a whole chromosome. Other studies have indicated the presence of centromeric alphoid sequences in 44-62% of micronuclei (CATALAN et al 1995). A higher number of centromere-positive micronuclei in older women (51.5%) than in younger women (34.3%) was documented. This finding is concordant with the results of FENECH and MORLEY (1985) who observed a kinetochore in 50% and 40% of micronuclei among elderly (age range 65-75 years) and young (age range 24-35 years) women, respectively.

Frequency of micronuclei

GUTTENBACH et al. (1994) reported an obvious increase in the frequency of micronuclei in individuals older than 70 years. Compared with young individuals (0.5-10 years), older donors display a three times higher frequency of micronuclei. In males, the number of nuclei with micronuclei increases from 0.2% to 0.48%. This difference is even more strongly expressed in females, with girls averaging 0.1% of nuclei with micronuclei, and with older donors having an incidence of 0.44% nuclei with micronuclei. This increase complements the data of FENECH and MORELY (1985), who have also found an approximately 4-fold increase in frequency of micronuclei in lymphocyte cultures from 80-year-old donors versus newborn donors. GANGULY (1993) reports 0.1-1.5% nuclei with micronuclei in males and 0.16-1.15% nuclei with micronuclei in female subjects.

HANDO et al. (1994) reported that 1% of binucleated lymphocytes from females ranging in age from 19 to 77 years, contained a single, two or three micronuclei and that 59% of these were kinetochore positive. This class of micronuclei definitely increases with age.

Sex-chromosome-positive micronuclei

The frequency of X-chromosome-positive micronuclei also increases with age. Among kinetochore-positive micronuclei, 77.3% contained one or more X chromosomes, while 64.9% kinetochore-negative micronuclei contained a signal representing the X chromosome. Among the binucleated cells scored from newborns, only 0.04% contained micronuclei and none of them had the X chromosome. These data suggest that micronuclei observed in adults are the result of their accumulation since birth (HANDO et al. 1994). The contribution of X-positive micronuclei among all micronuclei was significantly higher in the older age group (24%) than in the younger group (14%). In young females (<10 years), an average of 8% of the micronuclei have X chromosomes, but their proportion rises to 20% in old females (>70 years) (GUTTENBACH et al. 1994). Thus, the frequency

of micronuclei bearing a sex chromosome is about twice as high in donors older than 70 years than in donors younger than 10 years. Aneuploidy involving the X chromosome increases with age, resulting more frequently in losses by micronucleus formation than in gains, and involved the late replicating of X (GALLOWAY et al. 1978, ABRUZZO et al. 1985) (Figure 4).

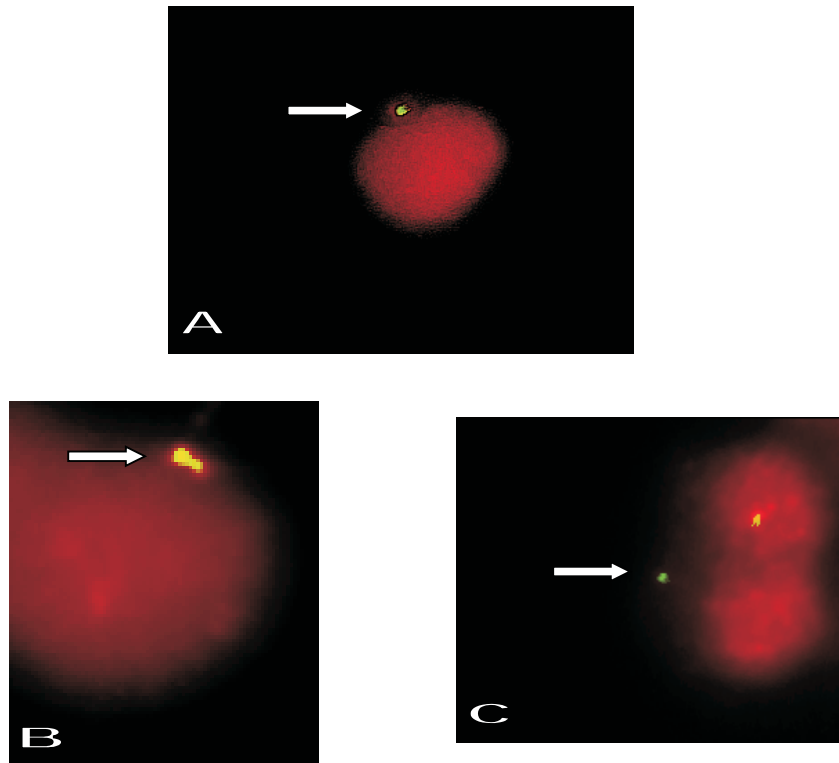


Figure 4. Sex-chromosome-positive micronuclei. FISH with X α satellite chromosome probe in lymphocytes from a male

A, B = mononucleated cell displaying X chromosome loss by micronucleus formation, C = binucleated cell displaying X chromosome loss by micronucleus formation. Arrowheads indicate the labelled X-centromeric region

In 1997 HANDO et al. found that healthy women ranging in age from 10 to 42 years, had a mean micronucleus frequency of 3.25 per 1000 cells; the same authors also described a tenfold difference in the frequencies of X-chromosome-positive micronuclei in females, as compared to males (HANDO et al. 1997). In males an average of 14% of the analysed micronuclei contained the Y chromosome, the frequency reaching up to 20% of the micronuclei detected in old males (GUTTENBACH et al. 1994). In general, studies of hyperdiploidy, hypodiploidy and reciprocal gain and loss by micronuclei formation have shown that

malsegregation of the X chromosome is common in lymphocytes of both men and women and more frequent than the Y chromosome malsegregation (CATALAN et al. 2000).

Autosome-positive micronuclei

CATALAN et al. (2000) found that the frequency of centromere-positive micronuclei was significantly higher among the older donors (51.5%) than among the younger donors (34.3%). When the contribution of the X and Y chromosomes was subtracted from the total of centromere-positive micronuclei, the resulting autosome contribution among all micronuclei was also found to increase significantly with age, from 20% of all micronuclei in the younger women to 28% in the older group. There is a 3.3- fold increase in the percentage of micronuclei that do not contain the X chromosome, although this increase is less significant than that of X-containing micronuclei (11-fold). This evidently demonstrates that both the X chromosome and autosomes are responsible for the age-dependent increase in micronuclei in women's peripheral lymphocytes (CATALAN et al. 1995).

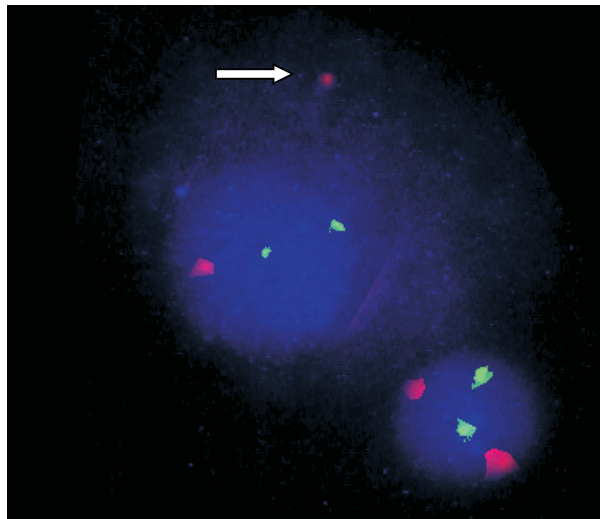


Figure 5. Autosome-positive micronucleus. FISH with 1 (red) and 6 (green) α satellite chromosome probe in lymphocytes: binucleated cell displaying chromosome 1 loss by micronucleus formation. Arrowhead indicates labelled centromeric region of chromosome 1

LEACH et al. (2001) showed that all of the 23 chromosomes could be present in micronuclei, although the X chromosome was seen most frequently. SHI et al. (2000) studied effects of age and sex on spontaneous malsegregation, nondysjunction and loss of chromosome 21 in human lymphocytes. Both males and females were grouped by age as 0-10 years, 20-30 years, 40-50 years, and 60-70 years. The loss of chromosome 21 was shown not to be age or sex re-

lated. A positive age-related increase in nondysjunction of chromosome 21 was found only in children and adult females (Figure 5).

Survival of aneuploid cells

Differential survival of various aneuploid cell types could produce a higher frequency of cells aneuploid for X than for any of the autosomes. There is apparently a continuous loss of autosomes by micronucleus formation, but these monosomic cells seem to have a selective disadvantage, most of them being unable to survive. This situation is likely to be similar for the early-replicating X chromosome, generally present in one copy in metaphases (RICHARD et al. 1994). The resultant 45,XX-A daughter cells will almost certainly die, while 47,XX+A cells may have a decreased chance of survival depending on the chromosome involved. In females, cell death resulting from nondisjunction is less likely to be a problem if the inactive X is involved. The resultant 45,Xa cell and 47,Xa Xi Xi cells (where Xa and Xi denote the active and inactive X chromosome, respectively) contain a single active X each and are likely to be fully viable. The presence of two Xi chromosomes in the 47,Xa Xi Xi cell may have a minimal (or no) effect upon survival because the proper dosages of genetic material have largely been maintained. If the active X undergoes nondisjunction, the 45,Xi cell will presumably die because there is no known mechanism by which an inactive X may become activated in somatic cells. The fate of the 47,Xa Xa Xi cell is less certain, but the gene dosage problems seen with autosomal aneuploidies suggest that these cells may experience diminished survival. No mechanism is known whereby a 47,Xa Xa Xi somatic cell can inactivate one Xa to become 47,Xa Xi Xi. Thus, while X chromosome aneuploidy would lead to a biased frequency of surviving cells, such a bias would be less pronounced than autosomal aneuploidy, which also suggests that X monosomies can exist *in vivo*. There are several reports of the X chromosome aneuploidy in various tumours.

Structural aberrations

The stable cytogenetic damage accumulates with age at a significantly higher rate than does unstable damage. Some findings show a significant increase with age: translocations and insertions (10-fold increase), dicentrics (3-fold increase) and acentrics (3-fold increase) (RAMSEY et al. 1995). The higher frequency of translocations, as compared to dicentric or acentric fragments, is consistent with their greater stability through cell division (TUCKER et al. 1999). VOROBTSOVA et al. (2001) reported on the increase in frequency of stable chromosome aberrations in lymphocytes as a function of donor age. No age dependence for dicentrics was observed. On the basis of these data it can be concluded that the frequency of stable chromosome aberrations is a reliable biomarker of ageing in humans.

Ageing is an adaptive programmed death mechanism and an inevitable result of accumulation of occasional injuries in an organism. It is suggested that injury is monitored by a special system sending a death signal to a death programme when the number of injuries reaches some critical level (SKULACHEW 2001). Although senescence might be detrimental as a potential cause of ageing, its evolutionary advantage is to provide an additional obstacle to the development of cancers (OUELLETTE et al. 2000). In the older population the use of gene therapy and apoptosis modulators and re-introduction of telomerase may have a therapeutic value in preventing the functional decline in the older population (JOAQUIN et al. 2001).

Acknowledgements. This review was prepared and original data presented here were obtained as a part of the project PBZ-KBN-022/PO5/1999 "Genetic and environmental factors of longevity" of the State Committee for Scientific Research (KBN), coordinated by the International Institute of Molecular and Cell Biology in Warsaw. Critical reading of the manuscript by Prof. Maria M. SAŚIADEK is gratefully acknowledged.

REFERENCES

- ABRUZZO M.A., MAYER M., JACOBS P.A. (1985). Aging and aneuploidy: evidence for the preferential involvement of the inactive X chromosome. *Cytogenet. Cell Genet.* 39: 275-278.
- BACKSCH C., WAGENBACH N., NONN M., LEISTRITZ S., STANBRIDGE E., SCHNEIDER A., DURST M. (2001). Microcell-mediated transfer of chromosome 4 into HeLa cells suppresses telomerase activity. *Gene. Chromosomes Cancer.* 31: 196-198.
- BERDANIER C.D., EVERTS H.B. (2001). Mitochondrial DNA in aging and degenerative disease. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 475: 169-184.
- BERTRAM M.J., BERUBE N.G., SWANSON X.H., PEREIRA-SMITH O.M. (1998). Assembly of a BAC contig of the complementation group B cell senescence gene candidate region at 4q33-q34.1 and identification of expressed sequences. *Genomics* 56: 353-354.
- BERTRAM M.J., PEREIRA-SMITH O.M. (2001). Conservation of the MORF4-related gene family: identification of a new chromo domain subfamily and novel protein motif. *Gene* 266: 111-121.
- BROWN T., FOX D.P., ROBERTSON F.W., BULLOCK I. (1983). Non-random chromosome loss in PHA stimulated lymphocytes from normal individuals. *Mutat. Res.* 122: 403-406.
- BRYCE S.D., FORSYTH N.R., FITZSIMMONS S.A., CLARK L.J., BERTRAM M.J., CUTHBERT A.P., NEWBOLD R.F., PEREIRA-SMITH O.M., PARKINSON E.K. (1999). Genetic and functional analyses exclude mortality factor 4 (MORF4) as a keratinocyte senescence gene. *Cancer Res.* 59: 2038-2046.
- CAMPISI J. (1996). Replicative senescence: an old lives' tale? *Cell* 84: 497-500.
- CATALAN J., AUTIO K., WESSMAN M., LINDHOLM C., KNUUTILA S., SORSA M., NORPPA H. (1995). Age-associated micronuclei containing centromeres and the X chromosome in lymphocytes of women. *Cytogenet. Cell Genet.* 68: 11-16.

- CATALAN J., SURRALLES J., FALCK G.C.M., AUTIO K., NORPPA H. (2000). Segregation of sex chromosomes in human lymphocytes. *Mutagenesis* 15: 251-255.
- COTTLIAR A.S.H., SLAVUTSKY I.R. (2001). Telomeres and telomerase activity. Their role in senescence and in neoplastic development. *Med. Buenos Aires*. 61: 335-342.
- COURT BROWN W.M., BUCKTON K.E., JACOBS P.A., TOUGH I.M., KUENSSBERG E.V., KNOX J.D.E. (1966). *Chromosome Studies on Adults*. London, Cambridge University Press.
- CROWE D.L., NGUYEN D.C., TSANG K.J., KYO S. (2001). E2F represses transcription of the human telomerase reverse transcriptase gene. *Nucleic. Acids. Res.* 29: 2789-2794.
- CUTHILL S., AGARWAL P., SARKAR S., SAVELIEVA E., REZNIKOFF C.A. (1999). Dominant genetic alterations in immortalization: role of 20q gain. *Gene. Chromosomes Cancer*. 26: 304-311.
- DECARY S., BEN HAMIDA C., MOULY V., BARBET J.P., HENTATI F., BUTLER-BROWNE G.S. (2000). Shorter telomeres in dystrophic muscle consistent with extensive regeneration in young children. *Neuromusc. Disord*. 10: 113-120.
- DELORME V.G.R., MCCABE P.F., KIM D.J., LEAVRE C.J. (2000). A matrix metalloproteinase gene is expressed at the boundary of senescence and programmed cell death in cucumber. *Plant. Physiol*. 123: 917-927.
- FENECH M., MORLEY A. (1985). The effect of donor age on spontaneous and induced micronuclei. *Mutat. Res.* 148: 99-105.
- FENECH M. (2000). A mathematical model of the in vitro micronucleus assay predicts false negative results if micronuclei are not specifically scored in binucleated cells or in cells that have completed one nuclear division. *Mutagenesis* 15: 329-336.
- FORD J.H., RUSSELL J.A. (1985) Differences in the error mechanisms affecting sex and autosomal chromosomes in women of different ages within the reproductive age group. *Am. J. Hum. Genet.* 37: 973-983.
- FOSSLIEN E. (2001). Mitochondrial medicine-molecular pathology of defective oxidative phosphorylation. *Ann. Clin. Lab. SCI.* 31: 25-67.
- GALLOWAY S.M., BUCKTON K.E. (1978). Aneuploidy and aging: chromosome studies on a random sample of the population using G-banding. *Cytogenet. Cell. Genet.* 20: 78-95.
- GANGULY B.B. (1993). Cell division, chromosomal damage and micronucleus formation in peripheral lymphocytes of healthy donors: related to donor's age. *Mutat. Res.* 295: 135-148.
- GUARENTE L. (1996). Do changes in chromosomes cause aging? *Cell*. 86: 9-12
- GUTTENBACH M., SCHAKOWSKI R., SCHMID M. (1994). Aneuploidy and ageing: sex chromosome exclusion into micronuclei. *Hum. Genet.* 94: 295-298.
- GUTTENBACH M., KOSCHORZ B., BERNTHALER U., GRIMM T., SCHMID M. (1995). Sex chromosome loss and aging: in situ hybridization studies on human interphase nuclei. *Am. J. Hum. Genet.* 57: 1143-1150.
- HAHN W.C., MEYERSON M. (2001). Telomerase activation, cellular immortalization and cancer. *Ann. Med.* 33: 123-129.
- HANDO J., NATH J., TUCKER J. (1994). Sex chromosomes, micronuclei and aging in women. *Chromosoma* 103: 186-192.

- HANDO J.C., TUCKER J.D., DAVENPORT M., TEPPERBERG J., NATH J. (1997). X chromosome inactivation and micronuclei in normal and Turner individuals. *Hum. Genet.* 100: 624-628.
- HORIKAWA I., PARKER E.S., SOLOMON G.G., BARRETT J.C. (2001). Upregulation of the gene encoding a cytoplasmic dynein intermediate chain in senescent human cells. *J. Cell. Biochem.* 82: 415-421.
- HORSMAN D.E., DILL F.J., MCGILLIVRAY B.C., KALOUSEK D.K. (1987). X chromosome aneuploidy in lymphocyte cultures from women with recurrent spontaneous abortions. *Am. J. Med. Genet.* 28: 981-987.
- JACOBS P., COURT BROWN W. (1961). Distribution of human chromosome count in relation to age. *Nature* 191: 1178-1180.
- JACOBS P. (1963). Change of human chromosome count distribution with age: evidence for a sex difference. *Nature* 197: 1080-1081.
- JACOBS P.A., BRUNTON M., COURT BROWN W.M. (1964). Cytogenetic studies in leucocytes on the general population: Subjects by ages 65 years and more. *Ann. Hum. Genet.* 27: 353-362.
- JOAQUIN AM., GOLLAPUDI S. (2001) Functional decline in aging and disease: a role for apoptosis. *J. Am. Geriatr. Soc.* 49: 1234-1240.
- KARAYIANNI E., MAGNANINI C., ORPHANOS V., NEGRINI M., MANIATIS G.M., SPATHAS D.H., BARBANTI-BRODANO G., MORELLI C. (1999). Transcriptional map of chromosome region 6q16-q21. *Cytogenet. Cell. Genet.* 86: 263-266.
- KIRSCH-VOLDERS M., FENECH M. (2001). Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. *Mutagenesis* 16:51-58.
- KLAPPER W., PARWARESCH R., KRUPP G. (2001). Telomere biology in human aging and aging syndromes. *Mech. Ageing. Dev.* 122: 695-712.
- KROEMER G., REED J.C. (2000). Mitochondrial control of cell death. *Nature Medicine* 6: 513-519.
- KUSKE M.D.A., JOHNSON J.P. (2000). Assignment of the human *PHLDA1* gene to chromosome 12q15 by radiation hybrid mapping. *Cytogenet. Cell. Genet.* 89: 1.
- LEACH N.T., JACKSON-COOK C. (2001). The application of spectral karyotyping (SKY) and fluorescent in situ hybridization (FISH) technology to determine the chromosomal content(s) of micronuclei. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 495: 11-19.
- LUDWIG B., BENDER E., ARNOLD S., HUTTEMANN M., LEE I., KADENBACH B. (2001). Cytochrome C oxidase and the regulation of oxidative phosphorylation. *Chembiochem.* 26: 392-403.
- MATTSON M.P., FU W.M., ZHANG P.S. (2001). Emerging roles for telomerase in regulating cell differentiation and survival: a neuroscientist's perspective. *Mech. Ageing. Dev.* 122: 659-671.
- MORELLI C., MAGNANINI C., MUNGALL A.J., NEGRINI M., BARBANTI-BRODANO G. (2000). Cloning and characterization of two overlapping genes in a subregion at 6q21 involved in replicative senescence and schizophrenia. *Gene* 252: 217-225.
- MUKHERJEE A.B., THOMAS S. (1997). A longitudinal study of human age-related chromosomal analysis in skin fibroblasts. *Exp. Cell Res.* 235: 161-169.

- MULLER F. (2000). The nature and mechanism of superoxide production by the electron transport chain: its relevance to aging. *J. Am. Aging. Assoc.* 23: 227-253.
- MULTANI A.S., OZEN M., NARAYAN S., KUMAR V., CHANDRA J., MCCONKEY D.J., NEWMAN R.A., PATHAK S. (2000). Caspase-dependent apoptosis induced by telomere cleavage and TRF2 loss. *Neoplasia* 2: 339-345.
- NEURATH P., DEREMER K., BELL B., JARVIK L., KATO T. (1970). Chromosome loss compared with chromosome size, age and sex of subjects. *Nature* 225: 281-282.
- NICHOLLS P., MARTIN J.M., KAHN J. (1978) A mathematical model predicting chromosome loss in cultured cells of young adults. *J. Theor. Biol.* 73: 237-245.
- NOWINSKI G.P., VAN DYKE D.L., TILLEY B.C., JACOBSEN G., BABU V.R., WORSHAM M.J., WILSON G.N., WEISS L. (1990). The frequency of aneuploidy in cultured lymphocytes is correlated with age and gender but not with reproductive history. *Am. J. Hum. Genet.* 46: 1101-1111.
- OUELLETTE M.M., SAVRE-TRAIN I. (2000). Telomere-controlled senescence. *M. S. Med. Sci.* 16: 473-480.
- PFEIFFER P., GOEDECKE W., OBE G. (2000). Mechanism of DNA double-strand break repair and their potential to induce chromosomal aberrations. *Mutagenesis* 15: 289-302.
- PINCHEIRA J., GALLO C., BRAVO M., NAVARRETE M.H., LOPEZ-SAEZ J.F. (1993). G₂ repair and aging: influence of donor age on chromosomal aberrations in human lymphocytes. *Mutat. Res.* 295: 55-62.
- PRESSL S., EDWARDS A., STEPHAN G. (1999). The influence of age, sex and smoking habits on the background level of FISH-detected translocations. *Mutat. Res.* 442: 89 - 95.
- RAMSEY M.J., MOORE D.H., BRINER J.F., LEE D.A., OLSEN L.A., SENFT J.R., TUCKER J.D. (1995). The effects of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. *Mutat. Res.* 338: 95-106.
- REED J.C. (2001). Apoptosis-regulating proteins as targets for drug discovery. *Trends Mol. Med.* 7: 314-319.
- RICHARD F., MULERIS M., DUTRILLAUX B. (1994). The frequency of micronuclei with X chromosome increases with age in human females. *Mutat. Res.* 316: 1-7.
- RONEN A., GLICKMAN B.W. (2001). Human DNA repair genes. *Environ. Mol. Mutagen.* 37: 241-283.
- SAMPER E., FLORES J.M., BLASCO M.A. (2001). Restoration of telomerase activity rescues chromosomal instability and premature aging in *Terc*^{-/-} mice. *EMBO*.
- SEMSEI I. (2000). On the nature of aging. *Mech. Ageing. Dev.* 117: 93-108.
- SHI Q.H., CHEN J.F., ADLER I.D., ZHANG J.X., MARTIN R., PAN S.J., ZHANG X.R., SHAN X.N. (2000). Increased nondysjunction of chromosome 21 with age human peripheral lymphocytes. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 452: 27-36.
- SKULACHEV V.P. (2001). The programmed death phenomena, aging, and the Samurai law of biology. *Exp. Gerontol.* 36: 995-1024.
- SMITH A., ELLIOTT G. (1980). Aneuploidy in culture. *J. Ment. Defic. Res.* 24: 159-165.
- STALLARD R., HANEY N.R., FRANK P.A., STYRON P., JUBERG R.C. (1981). Leukocyte chromosomes from patients of cytogenetically abnormal offspring: preliminary observations. *Cytogenet. Cell. Genet.* 30: 50-53.

- SURRALLES J., FALCK G., NORPPA H. (1996). In vivo cytogenetic damage revealed by FISH analysis of micronuclei in uncultured human T lymphocytes. *Cytogenet. Cell. Genet.* 75: 151-154.
- TUCKER J.D., SPRUILL M.D., RAMSEY M.J., DIRECTOE A.D., NATH J. (1999). Frequency of spontaneous chromosome aberrations in mice: Effects of age. *Mutat. Res.* 425: 135-141.
- VOJTA P.J., FUTREAL P.A., ANNAB L.A., KATO H., PEREIRA-SMITH O.M., BARRETT J.C. (1996) Evidence for two senescence loci on human chromosome 1. *Genes Chromosomes Cancer.* 16: 55-63.
- VOROBTSOVA I., SEMENOV A., TIMOFEYeva N., KANAYEVA A., ZVEREVA I. (2001). An investigation of the age-dependency of chromosome abnormalities in human populations exposed to low-dose ionising radiation. *Mech. Ageing. Dev.* 15: 1373-1382.
- WALLACE D.C. (2001). Mouse models for mitochondrial disease. *Am. J. Med. Genet.* 106: 71-93.
- WEIRICH-SCHWAIGER H., WEIRICH H.G., GRUBER B., SCHWEIGER M., HIRSCH-KAUFFMANN M. (1994). Correlation between senescence and DNA repair in cells from young and old individuals and in premature aging syndromes. *Mutat. Res.* 316: 37-48.
- WENG L.P., BROWN J.L., ENG C. (2001). PTEN induces apoptosis and cell cycle arrest through phosphoinositol-3-kinase /Akt-dependent and- independent pathways. *Hum. Mol. Genet.* 10: 337-342.
- WENGER S.L., GOLDEN S.L., DENNIS S.P., STEELE M.W. (1984). Are the occasional aneuploid cells in peripheral blood cultures significant? *Am. J. Med. Genet.* 19: 715-719.
- WHEELER W.J., CHERRY I.M., DOWNS T., HSU T.C. (1986). Mitotic inhibition and aneuploidy induction by naturally occurring and synthetic estrogens in Chinese hamster cells in vitro. *Mutat. Res.* 171: 31-41.
- ZAHN R.K., ZAHN-DAIMLER G., AX S., REIFFERSCHIED G., WALDMANN P., FUJISAWA H., HOSOKAWA M. (2000). DNA damage susceptibility and repair in correlation to calendaric age and longevity. *Mech. Ageing. Dev.* 119: 101-112.