Letters to the Editor

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Simultaneous decrease of telomere length and telomerase activity with ageing of human amniotic fluid cells

EDITOR—Telomeres are specific chromatin structures that cap chromosome ends and protect against chromosome degradation and end to end fusion.[1] As conventional DNA polymerases cannot fully replicate the ends of linear DNA, a progressive loss of telomeric sequences occurs in each round of DNA replication. Telomerase adds telomere repeats onto chromosome ends to overcome this end replication problem.[1] Telomerase activity is detectable in human germ cells, most immortalised cell lines, and in 80-90% of human tumour samples, in which the telomere length is preserved.[2] However, telomerase activity is not detected in most normal human somatic cells, with the result that telomere loss occurs with each cell division. After extended doublings, these cells enter a period of slow growth called senescence or crisis and stop dividing. This process may depend on critical telomere loss in one or a few chromosomes. The shortest telomere in a cell may also play an important role in oncogenesis.[2] Recently, low level telomerase activity has been detected in several human somatic cells, for example, lymphocytes, endothelial cells, hair follicle cells, colonic crypt cells, and basal layer cells of the epidermis.[3] In spite of this activity, telomeres shorten after each successive round of DNA replication. We report here the analysis of telomere length and telomerase activity in two cultures of human amniotic fluid cells (AL1 and AL2), showing a progressive decrease in both with ageing.

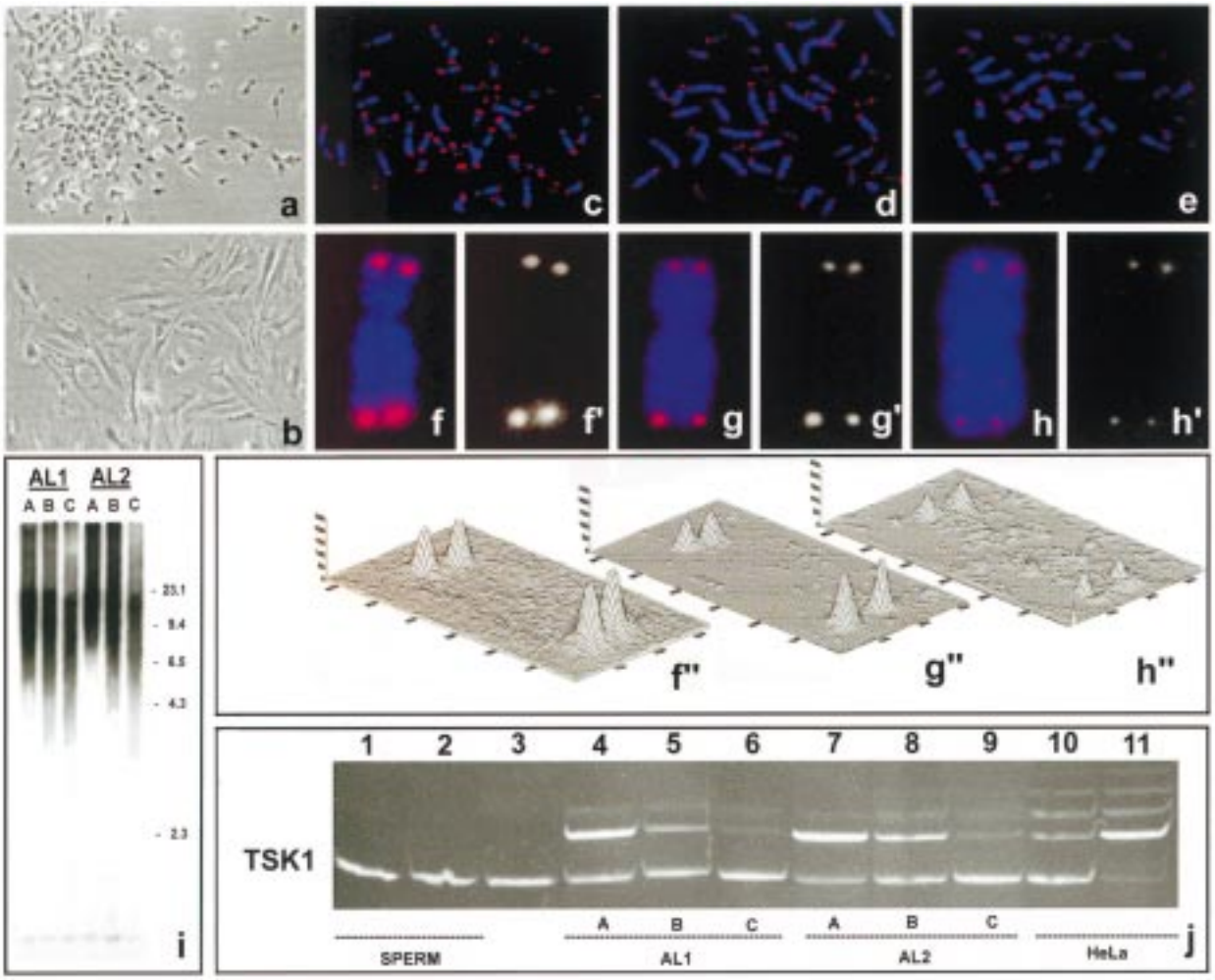

Figure 1 (Upper left) Cultured amniocytes at 14.7 MPD (a) and at 62.4 MPD (b) visualised under phase contrast microscopy. (Lower left) (i) Southern blot analysis of TRFs from amniocyte cultures AL1 and AL2 at MPD 14.7 (A), 42.7 (B), and 62.4 (C). (Upper right) FISH signals after hybridisation of a biotinylated telomeric probe detected using streptavidin-Cy3, with a round of signal amplification, from mitotic amniocytes at 14.7 (c), 42.7 (d), and 62.4 (e) MPD. Representative chromosomes with a pseudo-3D intensity profile showing strong (f, f', f"), medium (g, g', g"), and faint (h, h', h") telomeric FISH signals. (Lower right) (j) TRAP assay (Oncor) to assess telomerase activity. Lanes 1 and 2 correspond to extracts from 10 000 and 20 000 human sperm cells, respectively, used as a negative control. Lane 3 is a primer-dimer/PCR contamination control without cell extract. Lanes 4, 5, 6 and 7, 8, 9 correspond to amniocyte cultures AL1 and AL2 respectively at MPD 14.7 (A), 42.7 (B), and 62.4 (C). Extract from 10 000 cells was assayed for each experimental sample. Larger bands of the ladders appear very faint. Lanes 10 and 11 correspond to the activity of extract from 5000 and 10 000 cells, respectively, of a HeLa cell line used as a positive control. TSK1 is a template that amplifies a 36 bp internal control band semicompetitively with TRAP products.

Samples were obtained by amniocentesis at 14 weeks'gestation and subcultured over a period of 108 days. DNA extraction, chromosome spreads at metaphase, and cellular extracts were simultaneously performed in three stages. The first stage was 22 days after the initiation of culture (mean population doubling (MPD) 14.7), the second stage was established 64 days after the initiation of culture (MPD 42.7), and the final stage was 108 days after initiation of culture (MPD 62.4). The number of doublings between the first and second stage was 28, while between the second and third stage it was 19.7. The mean rate of cell division was 1.5 days, 1.5 days, and 6 days, at the first, second, and third stages, respectively.

Continuous growth of human amniotic fluid cells leads to replicative senescence. After accruing a number of population doublings they enter the terminally non-dividing state. This is also reflected in cell morphology, as shown in fig 1a and b, whereby telomere length progressively decreased after each round of DNA replication, as shown by Southern blotting of terminal restriction fragment (TRF) length (fig 1i). Mean TRF lengths were similar in both AL1 and AL2. They were estimated as 14 kb (SD 0.26), 11 kb (SD 0.23), and 8.4 kb (SD 0.25) at 14.7, 42.7, and 62.4 MPD, respectively. These TRFs were obtained by *Alu*I digestion and may be longer than standard TRFs produced by *Hin*fI + *Rsa*I digestion. Nevertheless, this implies a regular rate of telomere loss of around 100 bp per cell duplication, which is in accordance with data from lymphocytes,[4] for example. Whole telomeric quantitative digital image analysis of FISH signals per mitosis also confirmed this progressive decrease (fig 1c-h"). Around 33-37 mitoses were analysed per experimental point in each AL1 and AL2. The heterogeneity in the whole telomeric signal among mitotic cells was maintained through successive cell doublings (Levine test for homogeneity of variances, $p<0.05$). In contrast to TRF analysis, FISH suggests a non-linear decrease of telomeres with successive cell doublings. Thus, a decrease per doubling of 0.9% (AL1) to 0.6% (AL2) was obtained from 14.7 to 42.7 MPD, while it was 1.9 (AL1) to 3.9(AL2) times greater from 42.7 to 62.4 MPD. Discordance of FISH data with TRF analysis may depend on technical factors, for example, efficiency of probe hybridisation in FISH. Furthermore, while TRF study includes the whole population, FISH is restricted to mitotic cells. This selection could bias the estimate for the whole cell population. Another possibility is that while TRFs consist of both TTAGGG and degenerate sequences and non-TTAGGG subtelomeric sequences, FISH shows only the former.[5] In the case of a relatively greater decrease in pure telomeric sequences at the last MPDs, this could not be discriminated in the TRF analysis owing to the adjacent long non-telomeric sequences. The coefficient of variation from the whole telomeric FISH signal was between 21.5% and 31.2% in the amniotic fluid cell samples. This variability is much greater than the 13-15.4% observed after quantifying the FISH signals of alphoid DXZ1 and classical DYZ1 satellite DNA loci from one of the amniotic fluid samples. Unlike telomeres, these sequences do not vary in length among cells, so these latter coefficients must correspond to the variability of FISH intensity owing to technical factors.

Recently, several human somatic cell types like lymphocytes or colonic crypt cells have been shown to present a low level of telomerase activity which does not prevent telomere shortening. Accordingly, the human telomerase catalytic subunit gene is expressed in these cell types.[3] Though in a previous report primary cultures of human amniocytes did not show telomerase activity,[6] we detected this in both uncultured and cultured amniocytes from 14 weeks' gestation (data not shown) using the TRAP assay (Oncor). Telomerase activity at the initial stage of evaluation (MPD 14.7) was 34% (AL1) to 48% (AL2) of that of a HeLa cell line. Then it progressively decreased, the decrease per doubling being 2.9% (AL1) to 2.27% (AL2) from 14.7 to 42.7 MPD, while it was 0.86% (AL1) to 1.66% (AL2) from 42.7 to 62.4 MPD, which is 3.4 (AL1)-1.36 (AL2) times less (fig 1j). It is interesting to note the remarkable decrease from the first to the second stage, despite the mean rate of cell division having been similar at both stages. This suggests that, in this case, reduction in telomerase activity is not related to the growth rate, but to cell ageing, that is, the previous number of cell duplications. Another possibility is the simultaneous presence of different cell populations, given the presumed heterogeneity of amniotic fluid cells. Subpopulations with higher levels of telomerase activity were identified in cultured keratinocytes,[7] as well as in T and B lymphocytes.[8 9] Depending on the stage of gestation, amniocytes belong mainly to the amnion, skin, and the urogenital, respiratory, and digestive systems. Therefore, the decrease in telomerase activity with ageing of amniocytes could be related to a progressive reduction in subpopulations with high telomerase activity.

Slight but detectable telomerase activity was evident at the final stage (fig 1j). It has been suggested that telomerase activity is proliferation rate regulated.[10] For example, in quiescent confluent cultures of immortal cell lines[11] telomerase activity is repressed. So, the residual telomerase activity of aged amniocyte cultures possibly depends on a very small subpopulation of cells with duplication potential. At 62.4 MPD, most cells are quiescent and previous assays have shown that only one or two mean doublings took place during a further month of culture. Therefore, though the initial drop in telomerase activity in amniocyte cultures seems to be related to cell ageing rather than to duplication rate, near crisis cultures where mean rate of division is prolonged could decrease telomerase activity as a result of both cell ageing and proliferation rate. As argued above, persistence of a low cell number of a presumed telomerase active subpopulation could also explain this residual activity.

Overall, proliferating human amniotic fluid cells in culture decreased the length of telomere sequences, as shown by Southern blotting and FISH. In spite of telomere shortening, telomerase activity was detected by the TRAP assay. Nevertheless, this activity decreased as cell cultures aged, mainly in the initial cell doublings.

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45,X/47,XX,+18 constitutional mosaicism: clinical presentation and evidence for a somatic origin of the aneuploid cell lines

EDITOR—Constitutional mosaicism for two distinct chromosome aneuploidies is a rare cytogenetic abnormality. Usually in such cases, an autosomal aneuploidy is associated with a gonosomal aneuploidy. Little is known about the sequence of errors leading to such complex conditions. The only available studies addressing this issue concern three mosaic autosomal/gonosomal cases involving chromosome 8 (two cases) and chromosome 21 (one case), in all of which chimerism could be ruled out.[1 2] A mitotic origin was inferred for both mosaic trisomy 8 cases,[1] whereas the initial error in the trisomy 21 mosaic most likely occurred at meiosis.[2]

So far, trisomy 18 combined with monosomy X has been observed in three cases.[3–5] We have recently observed a fourth patient with mosaic monosomy X/trisomy 18. We report the clinical and cytogenetic characteristics and the results of molecular analysis, which was undertaken in order to determine the origin of the aneuploid cell lines.

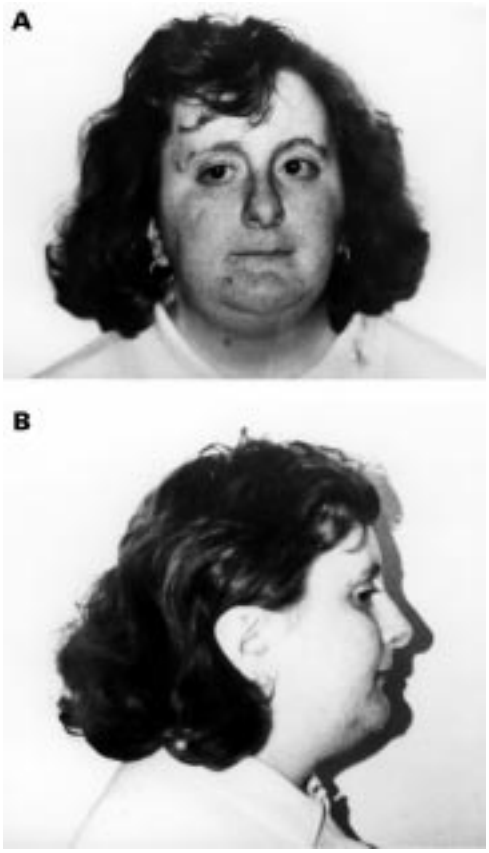

Figure 1 (A) Front and (B) lateral view of the proband aged 23 years 2 months. (Photographs reproduced with permission.)

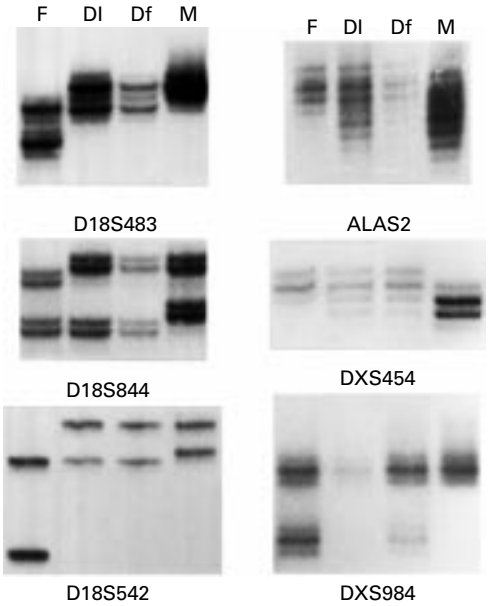

Figure 2 Typing of X chromosome and chromosome 18 microsatellite polymorphisms. The locus investigated is indicated below each autoradiogram. F=father, Dl=daughter, lymphocyte DNA, Df=daughter, skin fibroblast DNA; M=mother.

The female proband was the second child of healthy, unrelated parents. There was no family history of congenital anomalies or chromosome disorders. The father was 32 and the mother 34 years old at the time of her birth. She was born at term after an uneventful pregnancy and her birth weight was 3600 g. Psychomotor development was slightly delayed and she attended school up to the age of 14 years. Menarche occurred at 11 years, with regular menses up to 15 years. Thereafter, menses became progressively less frequent, until secondary amenorrhoea developed at 18 years of age.

The patient was referred for clinical and cytogenetic evaluation at the age of 23 years 2 months because of secondary amenorrhoea, mild mental retardation, and minor congenital anomalies. On clinical examination, height was 150.5 cm (3rd centile), weight 76 kg (>97th centile), and occipitofrontal circumference (OFC) 56 cm (50th centile). The face was asymmetrical, with a narrow nose and prominent nasal root and columella (fig 1). The ears were large and low set, with folded helices and large lobes (fig 1). The mouth was small, with a thin vermilion border and highly arched palate. There was bilateral cubitus valgus, brachydactyly, left Dubois sign, and abundance of white lines on both palms. Dermatoglyphics were (right) *t*, *abcd*, $L^{U}WWWW$ and (left) *t*, *abcd*, $L^{U}AAWW$. There were bilat-