THE PHENOTYPE OF FIBROBLASTS IN PROGERIA

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Progeria of Hutchinson-Gilford is a uncommon (1 per 8 million births) premature aging syndrome. It is the likely result of a sporadic autosomal dominant mutation. Death occurs during the second decade of life by cardiovascular alterations. Most of the connective tissues are affected (1).

We had the opportunity to investigate a new case of this disease. Fibroblasts cultures were established from two pieces of a skin biopsy taken in a 7 year old boy displaying typical clinical features of a Hutchinson-Gilford progeria. The cells collected from one set of explants (strain 1) displayed an unusual clustered growth pattern which was maintained even in cultures at high PDL while the cells collected from the second set of explants (strain 2) displayed a normal culture pattern. At low population doubling level (PDL≤ 10) cells divided actively and had an elongated shape as normal young fibroblasts. When reaching higher PDLs, the fibroblasts became progressively more polygonal, increased in size with a fibrillar appearance of the cytoplasm and ceased abruptly dividing at PDL 14 or 15 as classically observed in this disease (1). The biosynthetic activity in the two strains of progeria fibroblasts measured after a metabolic labeling with 3H-proline at PDL 5 was similar to that of two strains of normal young adult fibroblasts at identical passage in terms of collagen and non-collagen protein synthesis, except for an increased collagen deposition in the cell layer of the abnormally clustered strain 1. The pattern of the main labelled collagen polypeptides examined by SDS-PAGE was similar for the normal and progeria strains. However, immunofluorescence studies and Western-blot analysis revealed the presence, in the extracellular matrix of strain 1, of type IV collagen, a basement membrane collagen which is normally barely detectable in adult fibroblasts in monolayer cultures. By slot-blot hybridization a 40 times increase in the alpha 1 and alpha 2 type IV procollagen mRNAs level was recorded for strain 1 at low PDL by comparison to normal fibroblasts. Similarly, elastin mRNA level was also enhanced (10 times) as already reported by Sephès et al (2) while the steady-state level of the other tested mRNAs, alpha 1 (I), alpha 1 (III), alpha 1 (VI) procollagen, laminin and collagenase was not modified. At high PDL, the amount of these mRNAs was depressed by a factor of about 2 except for the alpha1 IV mRNA which remained unchanged. For strain 2 at high PDL, a lower increased level of the mRNA for alpha 1 IV, elastin and collagenase (200%, 150% and 120% respectively of the normal fibroblasts) was observed while the other tested mRNAs were depressed (50 to 95% of inhibition). It is worth noting that the expression of alpha1 (IV) and alpha2 (IV) collagen genes is high in fetal fibroblasts as compared to post-natal cells while little age-associated variation was observed for laminin mRNA level (3).
Similarly, the expression of the elastin gene also decreases as a function of age (4).

One may question the relatedness of the premature aging syndromes, Progeria (PHG), Werner's Syndrome (WS), and physiological aging (PA). While many common features are shared by these disorders as cardiovascular alterations and osteopenia, some distinct differences exist as patchy skin sclerosis in PHG and WS contrasting the classical skin atrophy observed in PA. A common finding in PHG and WS (5) is an increased urinary excretion of hyaluronic acid higher than in PA (1). It correlates with an altered turnover of glycosaminoglycans observed in culture of at least the WS fibroblasts (6).

Normal human fibroblasts in vitro display a finite lifespan inversely related to the age of the donor (7). Among other theories, fibroblasts aging in vitro is considered as a process of irreversible differentiation leading to ultimate cellular degeneration and death (8). The difference in the lifespan of mesenchymal cells in vitro between young and aged individuals could be related to the proportion in the explant of terminally differentiated cells (9). WS fibroblasts and even more PHG fibroblasts display a terminal morphotype after only a few doublings in vitro as well as a significantly reduced lifespan in vitro.

Progeric fibroblasts display a phenotype somewhat related to embryonic mesenchymal cells, a high elastin and collagen type IV expression together with an in vitro phenotypic and morphological characters of aged cells. The combination of these two traits suggests that a mutation in progeria could be responsible for an alteration of the program allowing embryonic mesenchymal cells to differentiate into post-embryonic cells.

References

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