FIBROBLAST PROLIFERATION AND COLLAGEN SYNTHESIS ACTIVATION
- ACTION IN THE CULTURE SUPERNATANT OF WERNER SYNDROME FIBROBLASTS -

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The Werner syndrome (WS) used as a model disease for aging displays some characteristics which differ from the simple promotion of aging based mainly on connective tissue abnormalities. In in vitro experiments, characteristics which have become apparent include the shortening of the life span of fibroblasts and the prolonging of the population doubling time, and more recently it has become known that increases in collagenase activity\(^1\), increases in the m-RNA levels of type I and III collagens\(^2\), and decreases in responses to PDGF and FGF\(^1\) are included. Here we demonstrate the effects of WS fibroblast culture supernatant on cell proliferation and collagen synthesis by dermal fibroblasts.

MATERIALS AND METHODS

We used 4 fibroblast strains (W42F, W43F, W38F and W57M) derived from WS patients' skin and 6 fibroblast strains (N20F, N33F, N41F, N43F, N54M and N64F) derived from control donors' skin. Fibroblasts were grown to confluency in 25 cm\(^2\) flasks. Fibroblasts grown in the culture flasks were washed with PBS and then cultured in DMEM with various concentrations of FBS for 48 hours. The culture supernatant was passed through a \(0.22\) \(\mu\) millipore filter and used as a conditioned medium. As the target cells normal human skin fibroblasts (N20F or N41F) were cultured in 12-well tissue culture plates. When cells became confluent, they were washed with PBS, and 1 ml of DMEM containing 5%FBS and 1 ml of conditioned medium was added and cultured for 48 hours. Also, culturing was carried out for 24 and 72 hours. At the end of this period, \(^3\)H-TdR (1 \(\mu\)Ci/ml) was added and then it was cultured for a further 3 hours. After washing with PBS, it was processed with 5\(\%\)TCA, dissolved in 1N NaOH, and a part of the radioactivity was determined with a liquid scintillation counter. The cell proliferation curve was also determined by inoculating \(5\times10^4\) normal fibroblasts onto a \(35\) mm dish, and adding a culture supernatant obtained from W42F and N41F immediately in a 48 hour culture, and further changing every two days. The number of cells was counted for 9 days. With regard to collagen and non-collagen protein synthesis, a culture supernatant was added to the target cells, and after culturing for 48 hours, the cells were washed 3 times with PBS.
Arakawa M et al: Characteristics of WS fibroblast and the culture medium was changed to DMEM supplemented with 50 μg/ml ascorbic acid, 50 μg/ml β-amminopropionitrile and 5 μCi/ml L-[2,3-3H]-proline; and after culturing for a further 3 hours, the medium was collected and the cell layers were harvested. The radioactivity of medium and cells together was measured after limited digestion with purified bacterial collagenase according to the method of Peterkofsky and Diegelmann 3) and divided into collagen-sensitive protein (CSP) and non-collagen-sensitive protein (NCSP).

RESULTS AND DISCUSSION

As shown in Figure 1, when the serum concentration was 0%, no difference was observed among cultures to which the WS fibroblast culture supernatant, the normal fibroblast culture supernatant and the medium (DMEM) only were added. When the serum concentration was 1, 5%, no difference was observed between the normal fibroblast supernatant and the medium, but the WS fibroblast culture supernatant brought about a marked increase in DNA synthesis of the target cells. The increase was observed at serum concentrations of 10% and 20% and increases occurred in the order of 5% > 1% > 10% > and 20%. With regard to the culturing time following the addition of the culture supernatant, the most marked effects were observed at 48 hours. In the cell proliferation curve, there was a clear increase in the number of cells to which the WS fibroblast culture supernatant was added in comparison with cells to which the normal fibroblast culture supernatant was added (data not shown). With regard to CSP and NCSP synthesis, both W42 and W43 fibroblasts strains displayed higher values than controls (Table 1). These results suggested that WS cells may excessively release growth factor-like substances.

REFERENCES