Growth Kinetics of Fibroblasts Derived from Normal Skin and Hypertrophic Scar

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In vitro growth kinetics of fibroblasts derived from normal skin and hypertrophic scar were performed using continuous ^{3}H-thymidine labeling method. In fibroblasts derived from normal skin, aging of the donor affects cell growth mainly by growth fraction (GF), but not labeling index (LI) and DNA synthetic time (Ts). When hypertrophic scar-derived fibroblasts are compared with normal fibroblasts, they showed a shorter Ts and lower LI and GF. This result suggests that in hypertrophic scar a small number of fibroblasts proliferate more actively, but most fibroblasts are non-growing cells. (Received May 5, 1987.)

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Hypertrophic scar is usually recognized as a reddened, elevated hard mass occurring as a consequence of deep injury to the skin. The most striking feature of hypertrophic scar is continuing build up of collagen over long periods of time (1, 2). Since fibroblasts actively synthesize collagen during the repair process of skin injury, it is suggested that these cells play a most important role in the development of scar tissue. In this culture study, we compared the growth kinetics of fibroblasts derived from normal skin and hypertrophic scar to clarify the mechanism of abnormal collagen formation in hypertrophic scar.

MATERIALS AND METHODS

Cell culture: Fresh skin specimens were obtained from twelve healthy patients ranging in age from 17 to 84 years (6 male and 6 female cases), and from seven patients with clinically confirmed hypertrophic scar in age from 4 to 46 years (male 6 cases, female 1 case). The biopsy specimens were cut into small pieces (1×1 mm) and a few pieces of skin were put onto a coverslip. Each coverslip was placed in a 35×15 mm plastic dish (Falcon Plastic, Oxnord, California) containing 2–3 ml Eagle's minimal essential medium (Nissui Seiyaku, Tokyo) supplemented with 10% fetal calf serum, and 60 μg/ml Epocelin (Fujisawa Seiyaku, Osaka). Cultures were maintained at 37°C in a highly humid incubator with mixture of 5% CO_2 in air. Culture fluid was routinely changed every three days. All experiments were carried out on 14–21 day-old cultures of exponential growth.

Cell labeling

After changing culture fluid, the cells were continuously labeled by the addition of 1 μCi/ml ^{3}H-thymidine (New England Nuclear, specific activity 24.7 Ci/mM). At 2, 6, 12, 24 and 48 h after labeling procedure, the cells were washed 3 times in cold phosphate buffered saline (pH 7.2), fixed in absolute methanol and air-dried. The labeled specimens were then processed for autoradiography. The labeling indices (LIs) were determined by counting the number of labeled nuclei per 500 cells. When there were 5 and more grains over the nucleus, the cell was identified as having a "labeled nucleus".

Estimation of growth parameters

Estimation of DNA synthetic time (Ts) and growth fraction (GF) was performed with continuous labeling method (3). During continuous labeling with ^{3}H-thymidine LIs arrive at the plateau level. Theoretically, the LI line is formed as shown in Fig. 1. The extrapolation of LI line back to zero labeling (Fig. 1) provides an estimated Ts. GF can be calculated from the time at which LI arrives at the plateau level. In the present experiments a 48 h LI was considered to represent GF, because Good (4) showed that GF was identical with the continuous LI for 48 h in human diploid fibroblasts.

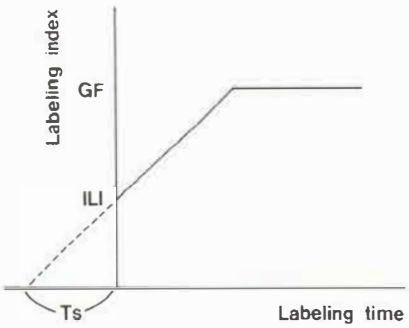

Fig. 1. Theoretical continuous labeling curve. Growth fraction (GF) is determined when a plateau level has been reached after labeling procedure. Initial labeling index (ILI) is theoretically estimated when the LI line is extrapolated to zero hour of labeling time. Ts = DNA synthetic time.

The comparative studies of growth kinetics of fibroblasts in vitro derived from normal skin and hypertrophic scar were evaluated statistically by Student's *t*-test.

RESULTS

Continuous LIs of fibroblasts derived from normal skin and hypertrophic scar were shown in Fig. 2. The patients from each category were separated into young (less than 40 years old) and old (more than 40 years old) groups. While some degree of variation exists in the LI obtained from each individual, there was a difference in the LIs of normal skin with respect to donor age. The fibroblasts derived from the young group (six patients: 17–32 years old) showed higher values in GF, but not in LI and Ts, than those from the old group (six patients: 49–84 years old) (Table I).

Compared with normal young fibroblasts, hypertrophic scar-derived fibroblasts showed a shorter Ts and lower ILI and GF when evaluated by a Student's *t*-test ($p<0.01$, $p<0.05$ and $p<0.025$) (Table I). This indicates that these fibroblasts proliferate more actively than those from normal young skin during the initial 24 h labeling, that is followed by the quiescent stage.

DISCUSSION

There is no agreement whether cultured fibroblasts derived from hypertrophic scar proliferate more actively than those from normal skin. In the present study, growth behavior of

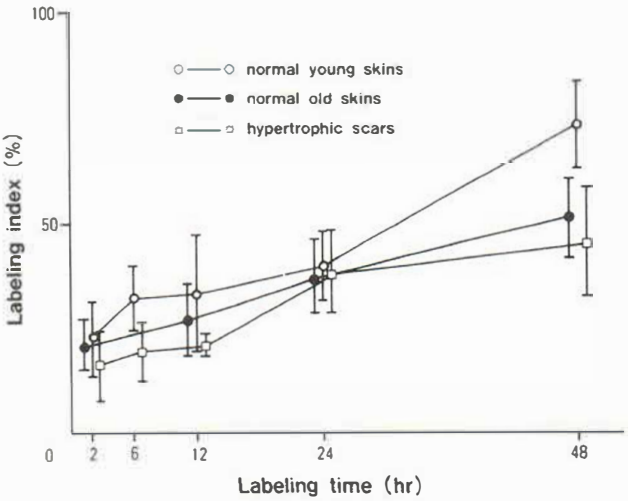

Fig. 2. Continuous labeling of normal skin and hypertrophic scars.

Table I. *Cell kinetics of normal skin and hypertrophic scars*

Initial labeling index (ILI), DNA synthetic time (Ts) and growth fraction (GF) were estimated. The means ± standard deviations for each group are presented

	ILI (%)	Ts (h)	GF (%)
Normal young skin (n=6)	22.9±8.9	24.4±11.7	73.1±10.3[a]
Normal old skin (n=6)	19.5±7.2	31.6±13.3	50.2±9.0
Hypertrophic scar (n=7)	12.4±7.7[b]	9.8±7.7[c]	55.2±13.1[d]

[a] $p<0.005$, compared with GF of normal old fibroblasts.
[b] $p<0.01$, compared with ILI of normal young fibroblasts.
[c] $p<0.05$, compared with Ts of normal young fibroblasts.
[d] $p<0.025$, compared with GF of normal young fibroblasts.

normal fibroblasts suggests that aging affects the cell growth mainly by reducing GF. Ristow et al. (5) have reported that human fibroblasts derived from biopsies have only a limited in vitro life span, varying from approximately 20 to 60 doublings according to the age of the donor. Klein et al. (6) have considered that GF is a most important parameter to estimate the growth kinetics of actively proliferating cells. On the other hand, LI and Ts may not usually exhibit the exact growth parameters of growing cells in vivo and in vitro (3, 7). Therefore, we confirm previous findings that dermal fibroblasts from normal skins proliferate actively acccording to the donor age.

The comparative study between normal skin and hypertrophic scar in this experiment is in contrast to the finding by Diegelmann et al. (8) in which growth curves between normal skin and hypertrophic scar-derived fibroblasts are not different. The difference may be attributed to the conditions of cell culture and methods estimating the growth kinetics. We suggest that a small number of the dermal fibroblasts in hypertrophic scar are cycling cells that proliferate more actively and rapidly, while increasing proportions of non-cycling cells to a greater extent occupy the scar compared with normal skin. An excessive accumulation of collagen in hypertrophic scar is ascribed to the increasing proportion of non-growing fibroblasts, because these cells are usually functioning cells expressing the characteristic differentiation.

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