Arch Dermatol Res (1986) 279:20–25

Archives of Dermatological Research

Comparative morphological and growth kinetics studies of human hair bulb papilla cells and root sheath fibroblasts in vitro

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Summary. Dermal papillae isolated from anagen hair bulbs obtained from biopsy specimens from five subjects with normal hair pattern, and fibroblasts derived from the mesenchymal root sheaths (RSF) of the same hair follicles were separately grown in culture and the cell-cycle distribution pattern on different days was analysed by applying DNA flow cytometry (FCM). Papilla cells (PC) exhibited distinctive morphological features by forming cell aggregates differing from RSF with respect to cell shape and growth pattern. They also proliferated remarkably more slowly than RSF. DNA-FCM analysis showed that both PC and RSF demonstrated synchronous fluctuations in the percentage of cells in $G_{1/0}$, S and G_2 + M phases during the period of subculture.

Key words: Hair bulb papilla cells – Root sheath fibroblasts – Cell culture – Growth pattern – DNA flow cytometry

Introduction

The vascularized hair bulb papilla is known to be important in both the induction of follicular development and the maintenance of hair growth, for supporting the metabolism of the rapidly proliferating matrix epithelium [3, 6, 7, 10–13]. Jahoda and Oliver [6] succeeded in establishing cell cultures of dermal papillae obtained from rat vibrissa follicles. Subsequently, they described the morphology of these cells in vitro, showing that rat papilla cells (PC), unlike dermal fibroblasts, exhibit a peculiar tendency to form aggregates [7].

Messenger [9] elaborated a method for the isolation of dermal papilla from the human anagen hair follicle and described some morphologic features of human PC in culture. However, no further studies have been devoted to human PC growth until quite recently (see Addendum). Last year we reported a study of the proliferation of cultured human PC performed using DNA flow cytometry (DNA-FCM) [8]. The present study was designed to elucidate the morphologic and proliferative behavior of human PC in vitro, compared with hair root sheath fibroblasts (RSF) of identical follicles in the same subjects. In addition, DNA-FCM investigation of subcultured PC and RSF was performed.

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Material and methods

Isolation of dermal papillae

Scalp biopsy specimens (5 × 20 mm) were obtained, with informed consent, from five patients (three females and two males aged 20–50 years, mean 37 years) undergoing surgery for intracranial aneurysms or neoplasms at the Department of Neurosurgery, University of Erlangen-Nürnberg [1]. Biopsies were uniformly taken from the occipital scalp region of patients suffering from neither male-pattern alopecia, nor from diseases affecting the hair and scalp. The biopsies were immediately placed in Dulbecco's modified Eagle Medium (DME; Gibco, Karlsruhe, FRG). The anagen hairs were meticulously dissected under a binocular microscope (Zeiss, Wetzlar, FRG; magnification × 16), transected 0.4–0.6 mm above the base, and transferred to a Petri dish containing the culture medium. Approximately 50 anagen hair bulbs were obtained from each biopsy specimen.

Isolation of the papillae was performed stereomicroscopically (magnification × 30) using two 20 G needles, each attached to a 1-ml syringe. The hair epithelium was separated from the surrounding reticular dermis and the dermal papilla gently extruded from the bulb with the tip of one needle while the other one was holding the bulb. After extrusion, the dermal papilla was transferred to the culture dish. Microscopic examination showed no residual epithelium left on the isolated papilla.

[1] Dr. M. Buchfelder is gratefully acknowledged for providing the scalp biopsies

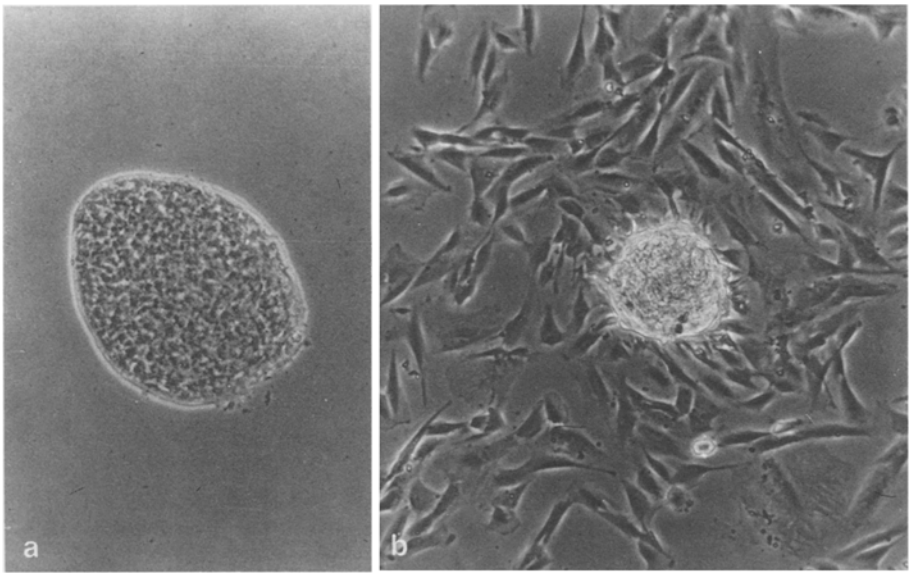

Fig. 1. a Dermal papilla isolated from a human anagen hair bulb (phase contrast), ×160. **b** Dermal papilla cells (PC) in culture after 10 days, showing spoke-like outgrowth of fibroblast-like cells (phase contrast) ×120

Cell culture establishment and maintenance

Papilla cells (PC). Seven dishes per proband (three explants each) containing 2 ml DME supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (0.584 mg/ml), and 20% inactivated fetal calf serum (all purchased from Gibco, Karlsruhe, FRG) were prepared in a humidified atmosphere (5% CO_2 at 37°C). The explants were left unobserved for the first 5 days, following which the medium was changed every 3rd day.

Root sheath fibroblasts (RSF). Fibroblasts were stereomicroscopically dissected from the hair follicle enveloping the mesenchymal sheath of the same scalp biopsy specimen. Small pieces were placed in 35-mm Petri dishes under coverslips and were cultured in the same manner as the hair bulb papillae. After 7 days the coverslips were removed and the medium was changed every 3rd day. The growing cells were maintained under these conditions for 4 weeks.

Subculture of PC and RSF. Having become confluent in primary culture after approximately 4 weeks, both PC and RSF were subcultured, using 0.25% trypsin and 0.02% EDTA solution (Gibco, Karlsruhe, FRG) to achieve single-cell suspensions. Petri dishes (35 mm in diameter) were seeded with 3×10^4 cells each; 28 dishes were prepared, 14 for each cell type. Subcultures were maintained in DME containing 10% inactivated fetal calf serum that was added to the medium.

After 24 h, two dishes were harvested from each source. The cells were counted using a hemocytometer, and submitted to flow cytometric DNA analysis. This procedure was repeated every 48 h using another two dishes from PC and RSF, for 15 days.

DNA flow cytometry (DNA-FCM)

After preparation of a single-cell suspension, nuclear DNA analysis of subcultured PC and RSF was performed as described previously [1, 4]. Briefly, the nuclear DNA was stoichiometrically stained with ethidium bromide and mithramycin [16] and quantitatively measured in a flow cytometer (ICP-22, Molter, Heidelberg, FRG). Cell-cycle-stage distribution analysis (i.e., calculation of the percentages of cells in the $G_{1/0}$, S, and G_2 + M phases of the cell cycle) was carried out using the method of Baisch et al. [2].

Results

Isolation and primary culture of hair bulb papillae

The dermal papillae could clearly be distinguished and isolated from the adjacent matrix epithelium (Fig. 1a). After 5–6 days in culture, the papilla explants became attached to the bottom of the dish and a few cells were seen sourrounding the explant in a spoke-like pattern (Fig. 1b). The cells resembled fibroblasts, with a flattened, irregularly outlined spindle shape, and often with faint striation in the cytoplasm. They continued to spread, but were arranged in round colonies after 3 weeks, forming monolayers at the periphery, and double or triple layers in the center. After 4 weeks, approximately, 5×10^4 cells were counted per papilla explant.

Subculture of PC and RSF

Morphology. In subculture, PC and RSF showed distinct morphological differences (Figs. 2a–c, 3a–c). PC were larger, flattened, and exhibited multiple short cytoplasmic processes (Fig. 2a). RSF were spindle-shaped with fewer, but longer, projections of the cell borders (Fig. 3a). After 3–4 days PC showed a further tendency to aggregate, forming clumps with large fibroblast-like cells at the periphery (Fig. 2b). In contrast to this, RSF grew in parallel alignment (Fig. 3b). This difference in growth pattern was maintained for each dish throughout the duration of

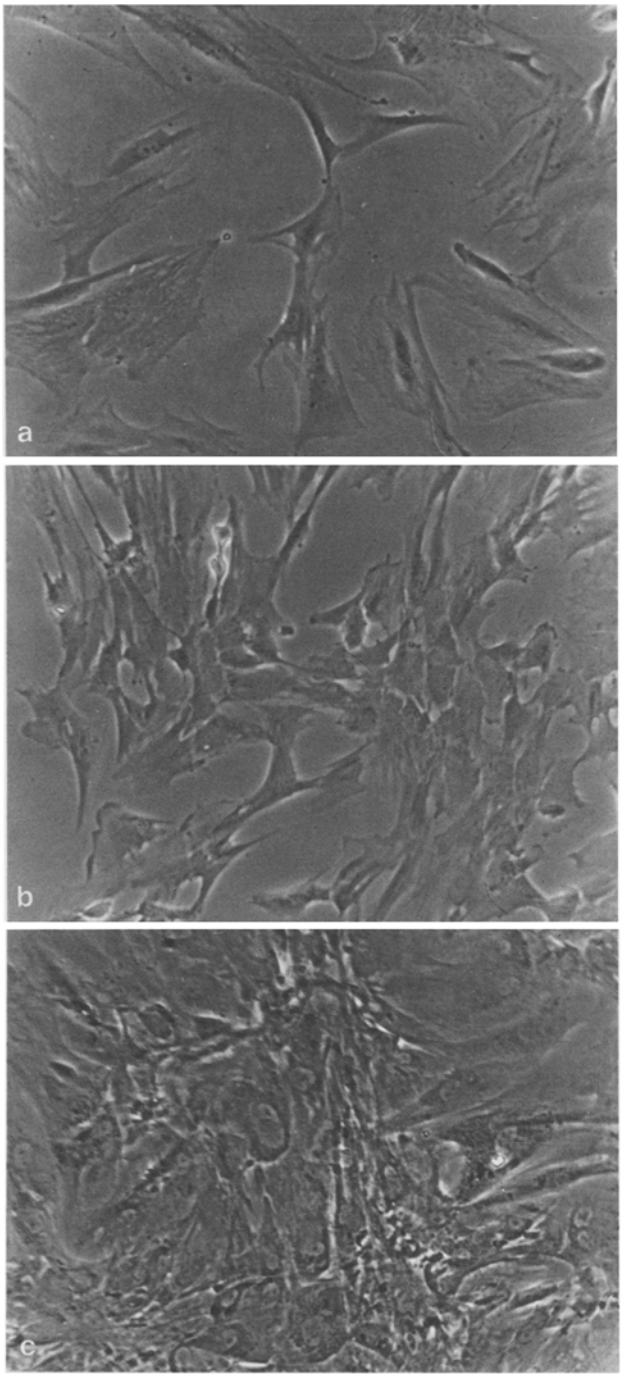

Fig. 2a–c. Morphology and growth pattern of papilla cells (PC) in subculture (phase contrast), ×120. **a** Large and flattened cells with multiple moderately long cytoplasmic processes after 2 days. **b** Tendency to form clumps after 8 days. **c** Clumps of rounded cells irregularly arranged in multiple layers after 14 days

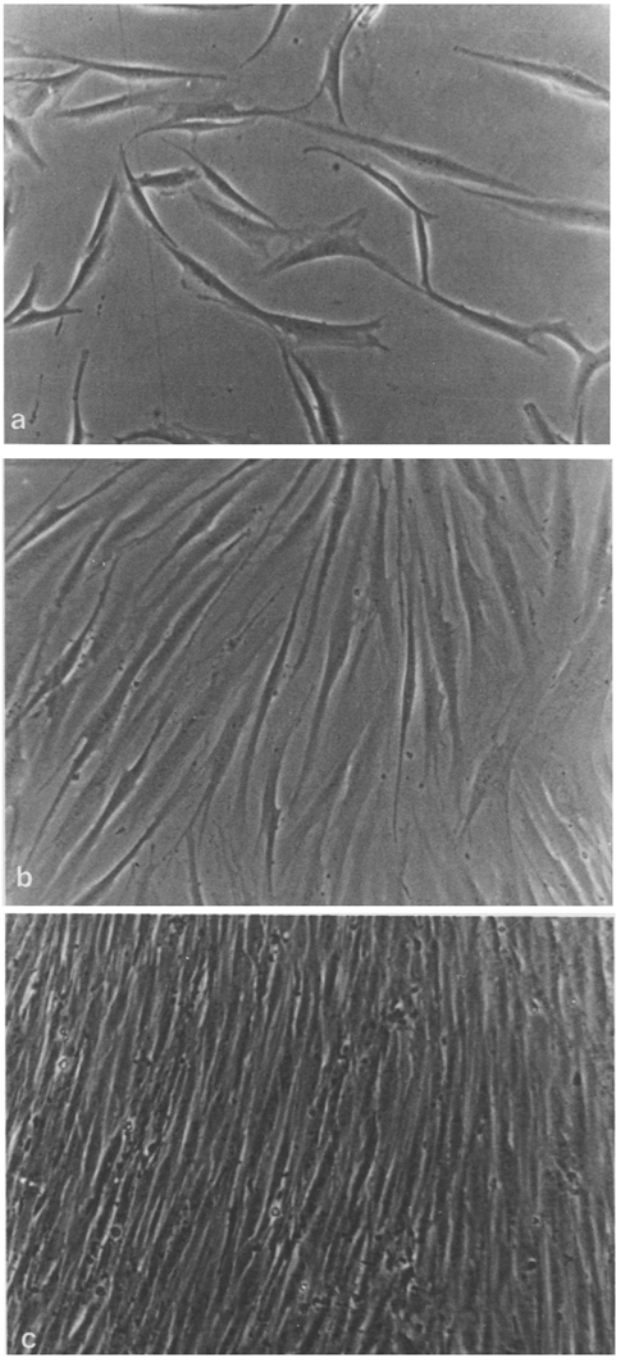

Fig. 3a–c. Morphology and growth pattern of root sheath fibroblasts (RSF) in subculture (phase contrast), ×120. **a** Typical spindle-shaped cells with long cytoplasmic projections after 2 days. **b** Parallel alignment after 8 days. **c** Storiform pattern after 14 days

culture. After 14 days, most of the PC were rounded, flattened, and arranged in irregular multiple layers (Fig. 2c). RSF continued to grow in parallel strands, sometimes forming a storiform pattern (Fig. 3c).

Cell population growth. Growth curves typical of each cell type are shown in Fig. 4. At the onset of the experiments there were approximately equal numbers of cells (2×10^4) from each source. Subsequently, PC

Table 1. DNA flow-cytometry (FCM) determined $G_{1/0}$, S, and G_2+M data (%) of papilla cells (PC) and root sheath fibroblasts (RSF) in subculture (mean values of five probands investigated)

	Days	1	3	5	7	9	11	13	15
PC	$G_{1/0}$	94.5 ± 0.6	73.0 ± 2.4	86.8 ± 1.3	83.9 ± 2.1	85.4 ± 1.2	87.6 ± 1.1	89.9 ± 1.4	82.8 ± 1.2
	S	2.1 ± 0.2	11.3 ± 1.6	3.1 ± 0.3	3.4 ± 0.2	5.9 ± 0.3	5.6 ± 1.3	3.1 ± 0.3	6.2 ± 0.8
	G_2+M	3.5 ± 0.3	15.8 ± 1.8	10.2 ± 1.7	12.7 ± 1.3	8.8 ± 1.5	6.6 ± 0.7	7.1 ± 1.3	11.0 ± 1.3
RSF	$G_{1/0}$	93.4 ± 0.4	66.9 ± 2.7	89.3 ± 1.1	86.7 ± 1.3	87.6 ± 0.9	82.2 ± 1.9	95.3 ± 1.3	93.4 ± 1.5
	S	3.0 ± 0.3	15.7 ± 1.9	3.1 ± 0.2	5.4 ± 0.4	6.9 ± 0.5	13.0 ± 2.1	1.8 ± 0.2	2.7 ± 0.5
	G_2+M	3.4 ± 0.2	17.3 ± 2.0	7.5 ± 0.9	7.9 ± 0.8	5.5 ± 0.8	4.8 ± 0.5	2.9 ± 0.9	3.9 ± 0.9

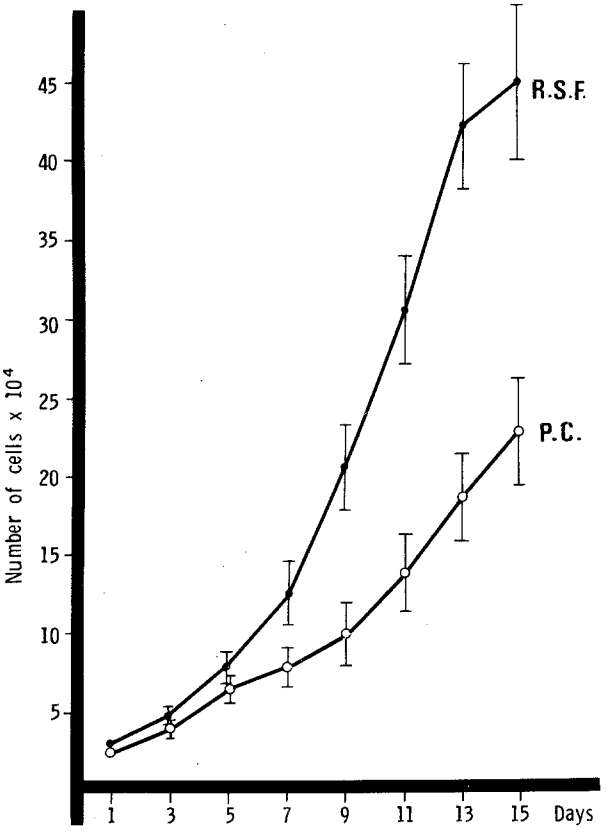

Fig. 4. Growth curves of PC (○) and RSF (●) in subculture. Petri dishes were initially seeded with 3×10^4 cells of either cell type

Fig. 5. DNA flow-cytometry (FCM) derived S-phase percentages of papilla cells (PC) (——) and root sheath fibroblasts (RSF) (– – –) in subculture. Synchronized fluctuations of S-phase values for both cell populations are shown

proliferated markedly slower than RSF, so that after 15 days the numbers of the latter were almost double those of PC.

DNA-FCM-derived proliferative data for both cell populations, as determined from five probands are shown in Table 1. PC and RSF displayed similar cell kinetics properties. For example, there were marked fluctuations in the percentages of cells in $G_{1/0}$, S, and G_2+M phases during the period of observation in both cell types. Maximal S and G_2+M phase activity was noticed on day 3 for both cell types, with minimal $G_{1/0}$ phase activity also occurring at the same time in both. The percentages of S phase activity for either cell type from one of the probands are shown in Fig. 5.

Discussion

Human hair bulb papilla cells (PC) show distinct morphologic characteristics when grown in vitro devoid of the influence of hair matrix epithelium [8]. It is their marked tendency to aggregate into cell clusters that is quite different from ordinary fibroblasts' growth pattern. The results of the present study conducted with human PC and RSF obtained from the

same scalp biopsy specimens are in good agreement with previous results for cultured rat vibrissa papillae [3, 6, 7].

It is tempting to conclude that the striking aggregative behavior of cultured PC reflects the role of these cells in the hair cycle in vivo, in providing a conformative structure for repeated reconstruction of the lower hair follicle. Thus, at the telogen arrest of hair growth with the supporting papilla no longer encapsulated by the matrix epithelium, intrinsic "constringent" behavior of the PC may prevent them from spreading into the surrounding mesenchymal tissue, thus preserving a stable histological structure for the renewal of the anagen hair [4]. This histoarchitectural stability may be due to a still-hypothetical intrapapillary cellular factor, since PC grew in clusters without concomitant induction by epithelium cells. Thus, our growth study yields evidence for the opinion submitted by Van Scott et al. [14] that papilla cells are more likely to induce than to retard increases in the proliferative capacity or volume of the matrix epithelium.

Some proliferative similarities of both cell types may be due to synchronization of cellular growth under the in vitro conditions prevailing at the onset of the subculture experiments. The synchronously high S-phase percentages determined by DNA-FCM on Days 3 and 11 support this hypothesis. However, the decrease as early as Day 11 indicates desynchronization.

It should be emphasized that the discovery of a higher percentage of cells in S phase, determined using either FCM or autoradiography, does not directly imply rapid tissue proliferation by itself, but also depends upon the duration of the different cell-cycle phases. Consequently, even in rapidly growing tissues low S-phase percentages can be obtained when the length of the S phase is short. Besides the morphologic differences of growth pattern in vitro, the divergent rates of proliferation of the two cell types was striking, since the numbers of RSF were nearly doubled those of PC after 15 days. Recently, differences in both the speed of proliferation and the collagen synthesis in vitro between fibroblasts of the papillary and reticular dermis have been reported [5, 15]. Papillary layer-derived fibroblasts showed faster growth in vitro than did reticular ones [5]. In our study, however, only fibroblasts adjacent to epithelium were investigated: hair papilla fibroblasts surrounded in situ by hair matrix cells and dermal fibroblasts derived from the mesenchymal root sheath. The divergent growth velocities of either cell population observed under in vitro conditions clearly indicate differences in the proliferative behavior of hair bulb PC and root sheath-derived fibroblasts.

Our study yields evidence that hair bulb PC, mainly consisting of fibroblasts and endothelial cells, represents a peculiar population of cells in the human skin, differing in their proliferative pattern from fibroblasts of the hair-follicle-enveloping connective tissue. It can be assumed that the distinct growth attributes of PC in vitro correspond to in vivo properties of the hair bulb papilla specialized for the support of both the development and maintenance of anagen hair production.

Addendum

Most recently, Messenger et al. reported some in vitro properties of dermal papilla cell lines established from human hair follicles (Br J Dermatol [1986] 114, pp 425). In their study, papilla cells (PC) were compared with fibroblasts of papillary dermis in culture. In our study, fibroblasts derived from the mesenchymal root sheath of hair follicles (RSFs) were used for comparison with PC, since RSF are in intimate proximity to epithelial hair follicle cells resembling the relationship between PC and matrix epithelium. Our results confirm those obtained by Messenger et al. with regard to the morphology and gross proliferative behavior of PC in subculture. Interestingly, RSF and papillary dermis fibroblasts showed similar growth rates.

Acknowledgement. Miss I. Schatz is gratefully acknowledged for skilled technical assistance.

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Received March 24, 1986