EPITHELIAL-FIBROBLASTIC ORGANIZATION IN CULTURES GROWN FROM HUMAN EMBRYONIC KIDNEY: ITS SIGNIFICANCE FOR MORPHOGENESIS IN VIVO

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SUMMARY

The morphological behaviour of explants of human embryonic kidney has been studied in order to investigate the rules governing interactions between epithelial and fibroblastic cells from the same tissue. When the fragments are cultured, epithelia migrate out first and are followed, a few days later, by cables which grow out from the fragments and which are composed of multilayered fibroblasts. These cables extend through the epithelia to reach the substratum, to which they adhere. The epithelia maintain an upper surface free of spread fibroblasts and are unable to multilayer, although occasional rounded-up cells adhere to this surface. Fibroblasts, however, not only multilayer in the cables but can act as a substratum for epithelial cells which migrate on the cable surface. Fibroblasts and epithelia from kidney thus follow the same behavioural rules that govern the interactions between kidney epithelia and fibroblasts from different tissues. The suggestion that these rules derive from tissue differences and that cells from the same tissue are more tolerant of one another is not borne out. These observations and those reported by others are interpreted in terms of the functional properties of the cells in vivo. It is further pointed out that only those epithelia that maintain a free surface in vivo would be expected to show this property in vitro. Finally, the implications of cells with essentially the same properties generating more than one structure are considered.

INTRODUCTION

The majority of studies on the interactions between epithelial and fibroblastic cells in vitro have shown that, once an epithelium has attached itself to a substratum, its free surface is not available for either fibroblasts or other epithelia to spread upon (Abercrombie & Middleton, 1968; Elsdale & Bard, 1974; Di Pasquale & Bell, 1974). An interesting partial exception to this rule has been reported by Visser, de Haas, Kox & Prop (1972): mouse mammary gland, when cultured in the presence of insulin and prolactin, gives rise to monolayer epithelial sheets with fibroblasts grouped into strands stretched across them; in the absence of the hormones, however, both cell types spread indiscriminately upon one another. A further exception has been reported by Brunette et al. (1977), who show convincingly that, in cultures of adult monkey periodontal ligament, epithelia and fibroblasts both multilayer and use one another as substrata. They, together with Pickett, Pitelka, Hamamoto & Misfeldt (1975), considered that the discrepancy between their observations and others may arise 'because it is possible that cultures derived from cells that are close neighbours
in vivo may be more tolerant of one another than those derived from widely separated tissues' (Brunette et al. 1977).

In this brief paper, observations on the interactions between the epithelia and fibroblasts that migrate from a single tissue, human embryonic kidney, are reported and it is shown that these cells act in accordance with the general rule rather than with the exceptions. Reasons for the discrepancy between different sets of observations are then discussed in the context of the functional properties of the tissue from which the cells are derived. Further organs whose tissues might also give anomalous results are considered and, finally, the significance of these observations in the general context of morphogenesis is discussed.

MATERIALS AND METHODS

Kidneys were obtained from the foetuses of 14 to 16-week therapeutic abortions; their capsules were removed and the tissue then minced with fine scalpels. Fragments were cultured in 60-mm Falcon dishes or on pieces of coverslip in F10 medium buffered with HEPES supplemented with 10 % foetal calf serum and antibiotics on a thrice-weekly medium changing regime (Elsdale & Bard, 1974).

Fixation procedures

Cultures for macroscopic examination were fixed in acetone—methanol (3:1, v:v) at —20 °C, and stained with May-Grunwald and Giemsa (Paul, 1975). Dishes were photographed with a Leitz macrophotography camera.

Cultures for examination in the scanning electron microscope were grown on coverslips 1 cm square, fixed for 2 h in 5 % buffered glutaraldehyde and for 40 min in osmium tetroxide, dehydrated with ethanol, transferred to amyl acetate and critical-point dried (Bard, Hay & Meller, 1975). They were then mounted on aluminium stubs and coated with gold in a sputterer (Polaron, London).

Cultures to be embedded were fixed and dehydrated as for the scanning electron microscope; they were then transferred to ethanol—Araldite (1:1, v:v) for 3 h and then through two 3-h changes of Araldite. The Araldite filled the dish to a depth of about 3 mm and was allowed to harden for 2 days at room temperature, 2 days at 37 °C and 2 days at 60 °C. The plastic was then removed from the dish with a scalpel and pliers leaving the cells in the basal layer of the Araldite disk. This surface of the Araldite was then reembedded to sandwich the cells.

Regions to be sectioned were selected under a dissecting microscope, cut out from the disk with a fine saw and the piece split at the required position. For this, the block was stuck to the base of a dissecting microscope with double-sided sticky tape and, under close examination, a razor was placed exactly above the site to be sectioned and the block was split by tapping the razor with a small hammer. The final block was placed in a chuck and trimmed; thick or thin sections were then cut. The former were stained with toluidine blue, the latter with uranyl acetate and lead citrate.

Microscopy

Live cultures were fitted with Cooper lids (Falcon) and filmed under phase-contrast optics in a 37 °C room on a Wild inverted microscope fitted with a Kodak ciné-special 16-mm movie camera and Wild time-lapse equipment. Lapse rates were 10–200 s.

Specimens for scanning electron microscopy were examined in a Cambridge S180 SEM. Thick Araldite sections were examined and photographed on a Zeiss Universal microscope. Thin sections were studied in an EMI EM 6B transmission electron microscope.
RESULTS

The growth of the culture

For the first 4 days after adhesion of kidney fragments to dishes, the cells that migrated out showed the behaviour and morphology of classical epithelial outgrowths: they formed monolayer pavements with ruffling membranes at their periphery (Elsdale & Bard, 1974). In filmed specimens, it could be seen that, where outgrowths met, ruffling ceased. Time-lapse films also showed that the cells in the monolayer were motile and glided slowly past one another away from the fragment or, if the fragment broke away from the substratum, they colonized the newly exposed plastic.

After these first few days a second group of cells started to leave the fragment: small outgrowths reached towards the substratum and put out processes as if to make adhesions. In specimens studied by time-lapse photography, many of these processes were seen to regress but a minority attached to the substratum and migrated away from the tissue apparently pulling more cells out of the fragment (no mitosis was observed in these cables). After 8 days (Fig. 1), the dish contained fragments, epithelial sheets and cables that extended from the fragments over the epithelium to the substratum. The number of such cables varied from none to 5 per fragment; the cables themselves could be thin (< 50 µm) or broad; in the latter case, they extended from perhaps half of the fragment and formed a sheet up to 0.5 mm wide.

It was possible to separate the epithelia from the cells of the cables. If cultures were trypsinized after about 5 days when only epithelial outgrowths were present and the fragments collected and then recultured, fibroblasts alone migrated out in typical radial outgrowths of loosely associated cells from a few fragments and in such cases neither epithelia nor cables were seen. It is therefore likely that the cables were composed of fibroblasts.

Observations on fixed material

In section, the cables were seen to come from the upper part of the fragment and could extend 1 mm or more from the fragment before they met the substratum (Fig. 2). Here, the cables penetrated the epithelial sheet and adhered directly to the substratum (Fig. 3). The cables themselves were often 8-10 cells thick (Fig. 3), the multilayering being characteristic of fibroblasts (Elsdale & Bard, 1974). Observations by transmission electron microscopy provided further evidence that these cells were fibroblastic in nature; they were thick, spindle shaped and had the rough endoplasmic reticulum characteristic of fibroblasts (Brunette, Melcher & Moe, 1977). No specialized adhesions were seen, but small amounts of fibrous material were present between the cells. Where the cables approached the substratum, it was possible to determine whether the fibroblasts made adhesions to the epithelial cells or to the plastic. Examination of several different cables showed that in all cases the cable extended through the epithelial sheet to the substratum. In cases where the cable made a shallow angle to the dish, fibroblasts and epithelia seemed to be in contact over several cell diameters before the cable penetrated the sheet; over this distance, however, there was no evidence of any adhesions between the 2 cell types.
When a section was cut through a region that appeared to be pavement epithelium, its appearance under the microscope confirmed the initial characterization. The cells were in flat monolayers with limited non-nuclear overlaps. Lateral cell margins were convoluted, with the cell membranes in close apposition; here, desmosomes were seen in thin sections. The only other evidence of multilayering was the presence of occasional rounded-up cells that were seen on top of the epithelium in time-lapse films, sections (Fig. 4) and in the scanning electron microscope (see later). The cells
had active processes and gave the impression that they were trying, but were unable, to spread. In thin section, no specialized adhesion could be seen between the rounded-up cells and the underlying epithelium although the membranes were in close (\( \sim 10 \) nm) apposition. It therefore seems that although spreading cannot take place, there are sufficient adhesions between the cells to prevent their separation during routine processing.

The surface appearance of cultures of kidney fragments as seen in the scanning electron microscope confirmed the impression given by sections. Epithelia (Figs. 5, 6) were flat and had raised nuclei and their cell boundaries were well defined. The kidney fragments were round and cellular organization could be seen on their surface; regions from which cables extended had no special features (Fig. 5). The cables themselves had smooth outlines and cell boundaries could not often be distinguished. While thin cables were particularly featureless, broad ones sometimes had corrugations parallel to the long axis and gave the impression that they were in tension (Fig. 6, arrow).

Where the cables met the substratum, they always appeared to penetrate the epithelial sheet. This was particularly noticeable when the cable was short and made a large angle to the substratum. Long cables that made a low angle to the substratum tended to have several regions of adhesion (Fig. 7) and the distance between the major adhesion and the end of cable was often more than 100 \( \mu \)m. In such cases, a gap was present between the cable and the adjacent epithelium (Fig. 8). Interestingly, on the upper side of such cables, epithelial cells often appeared to have migrated over the fibroblastic surface so that, when the specimen was turned in the microscope to expose this surface, it was hard to see where the cable ended and the epithelium began (Fig. 7). Although epithelial cells might spread on fibroblasts, no case was seen where fibroblastic cables adhered to or spread on epithelia attached to the substratum. In short, one surface of the epithelium appeared free of overlying, spread cells. As in sections, however, occasional rounded-up cells were seen on the epithelium (Fig. 9). Some of these may have been cells about to divide or, in the case of pairs, that had divided.

**DISCUSSION**

The main conclusion from the observations presented here is that epithelia from human embryonic kidney fragments interact with kidney fibroblasts in accordance with the same rules that govern their interaction with lung fibroblasts (Elsdale & Bard, 1974): that is, epithelia will not act as a substratum for the spreading or moving of fibroblasts or of further epithelial cells. Fibroblasts, in contrast, can act as substratum for themselves, as in the cables, and for epithelia which can migrate over the cables. The contention of Brunette et al. (1977) that cells from the same tissue may be more tolerant of one another is not borne out here. A possible clue to the discrepancies between their results and mine comes from the observations of Visser et al. (1972) on cultures of mouse mammary glands: here, the rules are obeyed if the cells are cultured with insulin and prolactin but not otherwise; indeed, the morphology of such
Fig. 5. A scanning electron micrograph of a kidney fragment (f) and outgrowth. The epithelial cells (e) form a flat pavement. From the fragment extends a broad cable (bc) and 2 narrower cables (c). Cellular outlines can be seen on the fragment and on the broad cable. ×160, scale bar = 100 μm.

Fig. 6. A scanning electron micrograph of a cable (c) meeting the substratum. It is not possible to see where the fan-shaped cable ends as epithelia have colonized the cable surface. Corrugations (arrow) can be seen on the cable. ×300, scale bar = 50 μm.

Fig. 7. The cable of Fig. 6 is shown rotated to display the several adhesions (e.g. arrow) that its fibroblasts make to the substratum. ×500, scale bar = 30 μm.

Fig. 8. A high-power scanning electron micrograph of the edge of a cable meeting the substratum (arrow). Epithelial cells (e) adhere to the substratum and to the upper side of the cable. There is, however, a gap maintained between the cable and the substratum-adhering epithelium. ×1000, scale bar = 10 μm.

Fig. 9. Scanning electron micrographs of rounded-up cells on spread epithelia. The pair of cells may just have divided. ×950, scale bar = 20 μm.
cultures in the presence of the hormones is very similar to that of the kidney cultures. Elsdale & Bard (1975) suggested that the tissue was functionally normal in the former but not in the latter case and that one would expect the normal rule to derive from the maintenance in vitro of morphogenetic properties that cells show in vivo. Brunette, Melcher & Moe (1976) have previously shown that the epithelial cells in periodontal ligament come from the Rests of Malassez which are derived from embryonic dental epithelia (Ten Cate, 1972). These cells, they further point out, have no known function in adult tissue. If this is so, it provides further evidence for believing that the rule given above applies only to functioning epithelia.

Brunette et al. (1977) further considered that, 'such controversy over the abilities of fibroblasts to spread on an attached epithelium may, in part, be caused by an oversimplified grouping together of apparently similar cells that can, in fact, exhibit diverse behavioural characteristics'. While these authors do not expand on what they mean by 'similar', it is well known that not all epithelia are equivalent either morphologically or functionally (for review, see Bloom & Fawcett, 1975). In turn, it is not unreasonable to expect that functioning and non-functioning or monolayered and stratified epithelia will show different properties in culture. The question is whether or not such distinctions are enough to predict that a spread epithelium can act as a substratum.

I suspect that the crucial property that will determine whether or not an epithelium in vitro can be substratum for other cells is the structure of that epithelium in vivo; if all of its cells have a free surface in vivo, they will maintain that structure under naturalistic conditions in culture (e.g. Visser et al. 1972); if not, then the epithelia will multilayer. The Rests of Malassez, for example, form epithelial clusters and strands of cells that are embedded in connective tissue; they have no free surface in vivo and, not surprisingly, this behaviour is carried over when the cells are cultured, as indeed was pointed out by Brunette et al. (1977).

There are many functional epithelia in tissues that multilayer or stratify. If the view expressed above is correct, then it is unlikely that such epithelia need maintain a free surface in vitro. In this context, at least 2 examples are known where epithelia that stratify in vivo also multilayer in vitro; adult skin (Flaxman, Lutzner & Van Scott, 1967) and adult human mammary gland epithelia (Flaxman & Van Scott, 1972). This multilayering should distinguish these epithelia from monolayer epithelia whose free surface in vivo would be expected to be maintained in vitro. It was the original intention of Elsdale & Bard (1974) to find that particular property that allowed these latter epithelia to maintain a free surface in vivo and so be able to generate a range of cellular organizations. They showed that the surface of these epithelia remained free because other cells were unable to spread upon it. While their observations on the interactions of fibroblasts and epithelia permitted the genesis of several tissues to be considered, it is now clear that the net was too loosely drawn and that epithelia should not be expected to maintain a free surface in vitro if they do not do so in vivo.

Epithelial and fibroblastic cells can display a considerable range of forms. In the case of the kidney, the original structure was of epithelial tubules in a mesenchymal matrix, the whole being enclosed in a capsule. Removal of this capsule and the
availability in vitro of a flat substratum and ubiquitous food for the cells resulted in the formation of a new structure comprising epithelial sheets and fibroblastic cables. It was not, however, changes in the cell properties that dictated the transition but the altered nature of the environment. It is not difficult to see how, in embryos, such geometric considerations can also play a role in organogenesis. Consider 2 ways in which initially separated epithelia and fibroblasts with the properties of the kidney cells may interact: let motile epithelia be able to invade the mesenchyme in the first case but not in the second. In the former example, tubules and vesicles will be expected; in the latter, a bounding membrane will emerge (Elsdale & Bard, 1974). If, in the former case, the epithelia can also multilayer, epithelial 'rests' such as those of Malassez would be likely to form. These simple examples show that, in order to explain any particular instance of morphogenesis, it is necessary to understand not only the properties and interactions of the cells but also the geometric constraints on them.

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REFERENCES


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