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ALTERED RABBIT ENDOTHELIAL CELL PHENOTYPIC EXPRESSION IN RESPONSE TO HUMAN FIBRONECTIN

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SUMMARY: When rabbit corneal endothelial cells were cultured in excess concentrations of human fibronectin an altered phenotypic expression was observed. Cell appearance was changed radically and collagen synthesis was specifically inhibited in a dose-response fashion. This study provides further evidence that fibronectin may be one of the developmental signals which act at a molecular level and is capable of interspecies activity.

INTRODUCTION: While many aspects of the process of cellular differentiation of pluripotent cells have been elucidated, the precise nature of the signals in many developmental processes remain to be determined. Early studies of the induction phenomenon indicated that certain cells could induce a specific pattern of differentiation in proximate, but distinct, cell types (1). Further investigation revealed that a porous barrier between the two distinct types of cells did not prevent this inductive influence, indicating that rather than being an exclusively physical phenomenon, a freely mobile chemical messenger must be contributing in a substantive way to this inductive effect (2). Moreover, cells from one species were capable of inducing differentiation in cells obtained from another species (3).

More recent evidence indicates that fibronectin, a glycoprotein found in blood and associated with cell surfaces, may provide such a signal for differentiation. It has been demonstrated that fibronectin plays a key role in the attachment and motility of cells (4). Thus, fibronectin may be involved in the morphogenetic movement of various cell types to their respective final destinations in the developing embryo (5-6). As various cell types develop, they show alterations in cell surface fibronectin which correlates with the

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onset of cytodifferentiation (7). Cells undergoing transformation also undergo substantial alterations in their cell surface fibronectin (8). Recently, studies have demonstrated that exogenous fibronectin is capable of altering the phenotype and cellular metabolism of chick chondrocytes *in vitro* (9-10). The present work extends these observations to corneal endothelial cells and indicates that this signal for altered differentiation is capable of interspecies communication, underscoring its possible universality as a signal of induction and differentiation.

MATERIALS AND METHODS: Fibronectin was purified from human plasma by gelatin-sepharose chromatography, the purity being verified by SDS-gel electrophoresis and amino acid analysis (11). Corneal endothelial cells were obtained from rabbits and maintained in culture as previously described (12). After the second passage, when the cells reached confluency, all cultures were labeled with Dulbecco's modified Eagle's medium with 10% fetal calf serum, 50µg/ml gentamycin, ascorbic acid (100µg/ml), beta-aminopropionitrile (50µg/ml) and (^{3}H)-proline (12.5µCi/ml). In the first series of experiments, at this same time, cultures received 100µl of either supplemented medium without labeled proline, purified fibronectin (1 mg/ml), or 4 M urea (as the purified fibronectin was maintained in 4 M urea to prevent adhesion of the fibronectin to laboratory glass/plasticware). At intervals of 24, 48, 72 and 96 hours after the addition of the labeled medium, culture medium was harvested and assayed for collagen content by a modified procedure of pepsin digestion and perchloric acid precipitation (13). Cell number in the culture dishes was determined by fluorometric analysis of DNA (14). In the second series of experiments, the same general procedure was followed but cultures were supplemented with 50, 100, 150 and 200µl of fibronectin, 4 M urea or additional unlabeled medium. Collagen and DNA contents were assayed as above. Fibronectin concentrations of individual cultures were ascertained by enzyme-linked immunoassay (ELISA) (15). All statistical analyses were performed by students t-test or two-way analysis of variance (16).

RESULTS AND DISCUSSION: When human fibronectin was added to rabbit endothelial cells in culture, by 48 hours a dramatic alteration in cell phenotype was observed. Instead of the expected polygonal morphology, as was seen in control and urea-treated cultures, endothelial cells treated with fibronectin pulled back from one another and developed multiple processes, possessing a fibroblast-like appearance. A similar change in cell morphology was seen in chick chondrocytes cultured in high concentrations of chick fibronectin (9). Such morphological changes were reversible upon removal of the exogenous fibronectin.

There was a concomitant alteration of macromolecular synthesis and/or secretion by rabbit endothelial cells treated with human fibronectin. Fibronectin-treated cultures experienced a progressive decline in collagen production, as reflected by the total collagen content of the culture medium (Figure 1).

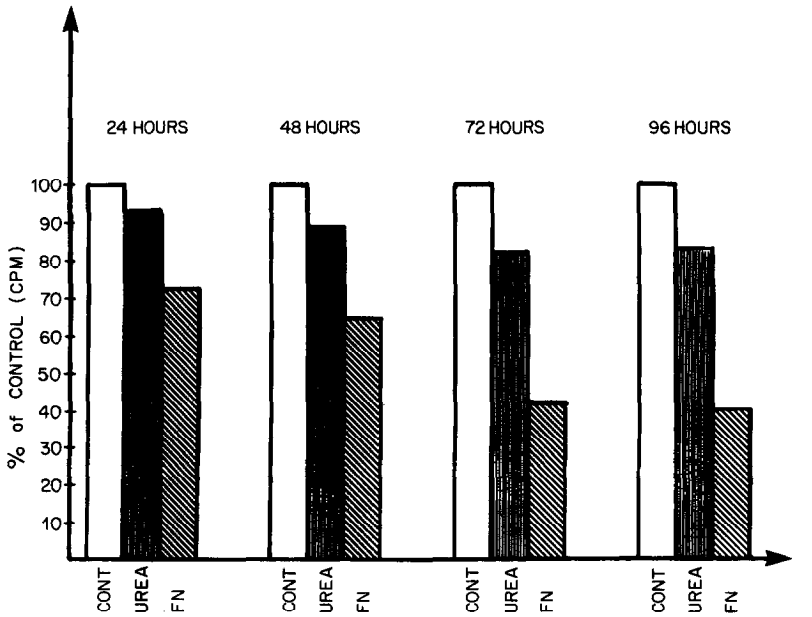

Figure 1: Total amount of collagen synthesized and secreted into the extracellular medium at 4 sequential time periods, expressed as a percentage of that synthesized and secreted by control cultures at that time interval (cont=control cultures; urea= urea-treated cultures; FN= fibronectin-treated cultures).

For instance, at 48 hours, endothelial cells treated with 100µl of fibronectin had produced only 64% as much collagen as had control cultures. By 96 hours, similar cultures treated with 100µl fibronectin contained only 40% as much collagen as did control cultures. The 4 M urea also had an effect upon collagen production, but this was relatively minor when compared with the influence of fibronectin, per se. The decrease in collagen production was not due to a decline in cell number, as total DNA content of the various cultures did not differ significantly. Moreover, it was not merely a reflection of altered synthesis of total protein, as while total protein synthesis was also decreased, collagen production was decreased to approximately a three-fold greater extent. The decrease in collagen concentration in the medium may have been the result of either reduced de novo synthesis of collagen, decreased intracellular processing and secretion of collagen or it may reflect a change in the type of collagen synthesized. Studies are currently underway to investigate these phenomena.

The precise level of fibronectin was also important in determining the degree of alteration in cellular phenotypic expression. Analysis by ELISA

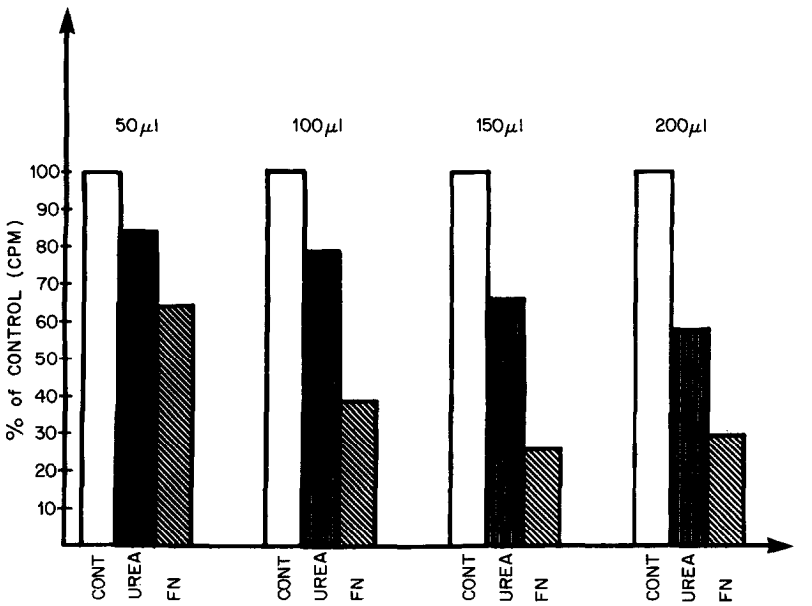

Figure 2: The influence of increasing levels of fibronectin upon total collagen synthesis and secretion at 96 hours of culture (cont= control cultures; urea= urea-treated cultures; FN=fibronectin-treated cultures).

indicated that the original medium contained approximately 80µg/ml fibronectin, the cultures containing 50µg exogenous fibronectin had a concentration of 130µg/ml, and those containing 100µl added fibronectin had a concentration of approximately 180µg/ml. As the amount of fibronectin which was added to cultures was increased from 50 to 150µl, the magnitude of the influence upon collagen synthesis was increased as well (Figure 2). At 96 hours of culture, 50µl of exogenous fibronectin caused a 36% reduction in collagen production, whereas 100µl and 150µl of exogenous fibronectin resulted in a 62% and a 74% reduction, respectively, in total collagen synthesis and/or secretion. However, beyond the level of 150µl of added fibronectin, there was no further reduction in total collagen production noted. Collectively, these results indicated that under these culture conditions a dose-responsiveness was experienced between 50 and 150µl of added fibronectin, but beyond this limit, no further effect upon collagen production was noted. Such graded responsiveness lends further support to the hypothesis that fibronectin may play a key role in differentiation and development. Recent studies involving rapidly dividing chick chondrocytes treated with chick fibronectin also responded with an alteration

in both the amount and type of collagens and glycosaminoglycans produced (9). The present work indicates that cells which are not dividing rapidly are also susceptible to this influence and it is active on an interspecies basis.

Components of the extracellular matrix, fibronectin prominent among them, have been suggested as critical factors in cellular differentiation and resultant morphogenesis (17). Recent observations extend this hypothesis to the earliest stages of embryogenesis (18). The pattern of deposition in the extracellular matrix may be important in stimulating the motility of various cell types and in directing their migration in the embryo (19-20). Fibronectin is found in a wide range of species and biochemical and immunological analyses indicate that they are remarkably similar (21). Fibronectin is also known to interact with a variety of different substances, including collagen, fibrin, glycosaminoglycans and cell surface lectins, underscoring its versatility in any developing system (6). Moreover, in adult organisms, fibronectin is found to a varying extent on large number of cell types including fibroblasts, myoblasts, hepatocytes, endothelial cells, intestinal epithelial cells, and astroglial cells; a soluble form is also present in the plasma and aqueous humor (11, 21-26). It is entirely conceivable that the precise level of fibronectin, or an absolute lack of fibronectin in certain cell types, may influence the degree of cell differentiation in the developing embryo and may also be necessary to maintain the differentiated state in mature organisms. Fibronectin may also be an important regulatory influence in processes such as wound healing and tissue regeneration, which require alterations in the state of differentiation of individual cell types within the mature organism. Local concentrations of fibronectin have been shown to change at the wound site (27); alterations in the collagen synthesis such as those demonstrated by the present experiment would be essential for normal wound healing. While the control of development in embryonic systems is, no doubt, a multi-faceted phenomenon, it is quite plausible that fibronectin may play an important role in modulating the interaction of various cell types.

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