ENDOTHELIAL GROWTH FACTORS AND EXTRACELLULAR MATRIX REGULATE DNA SYNTHESIS THROUGH MODULATION OF CELL AND NUCLEAR EXPANSION

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SUMMARY

Studies were carried out to analyze the mechanism by which extracellular matrix (ECM) molecules and soluble growth factors interplay to control capillary endothelial cell growth. Bovine adrenal capillary endothelial cells attached to purified matrix components but spread poorly and exhibited low levels of DNA synthesis in the absence of exogenous growth factors or serum. Addition of cationic, heparin-binding growth factor purified from either human hepatoma cells or normal bovine pituitary (fibroblast growth factor) induced extensive cell spreading and up to eight fold increases in DNA synthetic rates relative to levels observed in cells on similar substrata in the absence of mitogen. However, the extent of this response differed depending upon the type of ECM molecule used for cell attachment (fold increase on type III collagen > gelatin > type IV collagen > fibronectin > type V collagen >>> laminin). Computerized morphometry demonstrated that endothelial cell DNA synthetic rates increased in an exponential fashion in direct relation to linear increases in cell and nuclear size (projected areas). Similarly sized cells always displayed the same level of DNA synthesis independent of the type of ECM molecule used for cell attachment or the presence of saturating amounts of growth factor. In all cases, DNA metabolism appeared to be coupled to physical expansion of the cell and nucleus rather than to a specific cell morphology (e.g. polygonal versus bipolar). These findings suggest that ECM may act locally as a “solid state” regulator of angiogenesis through its ability to selectively support or prohibit cell and nuclear extension in response to stimulation by soluble mitogens.

Key words: fibroblast growth factor; cell shape; nuclear shape; signal transduction; angiogenesis.

INTRODUCTION

A great deal of progress has been recently achieved in the search for hormones that control angiogenesis. Soluble factors have now been purified from both normal and neoplastic tissues which can induce neovascularization in vivo and stimulate endothelial cell proliferation in vitro (3,27,42). However, the growth-promoting action of angiogenic factors is alone not sufficient to explain the generation of higher order tissue architecture. Repeated endothelial cell divisions would result in production of a disorganized pile of cells in the absence of additional regulatory controls. Indeed, development of branching capillaries and other specialized vascular structures is made possible through establishment of local differentials in endothelial cell proliferative rates in the midst of active angiogenesis (i.e., in the presence of high concentrations of soluble endothelial mitogens)(12). Thus, to understand the basis of angiogenic regulation, we must first dissect the mechanism by which endothelial growth factor action is selectively prohibited or supported locally.

Extracellular matrix (ECM) molecules are good candidates for local regulators of capillary development. In the embryo, tissue-tissue interactions that guide histogenesis are transduced over intervening ECM (17) and, in certain tissues, pattern formation appears to be linked to directed alterations of ECM turnover (1,45). Regional variations of ECM composition and integrity are observed during vascular development (13,23) and past studies have shown that purified ECM components can modulate the growth and organization of capillary endothelial cells in vitro (36). Yet, the mechanism by which ECM and soluble...
growth factors interact to control capillary endothelial cell growth remains unknown.

In the present study, bovine adrenal capillary endothelial (BCE) cells were plated on purified ECM components (laminin, fibronectin, types III, IV, & V collagens) or gelatin and exposed to purified human hepatoma-derived growth factor (HDGF) or basic fibroblast growth factor (FGF) isolated from normal bovine pituitary gland. HDGF and FGF are members of a newly described class of cationic, heparin-binding growth factors which share sequence homology, antigenic sites, and the ability to stimulate angiogenesis in vivo (7,29,32). This investigation was also carried out in the absence of serum so that we could determine effects that resulted solely from interactions between cells and distinct matrix molecules. We now show that the mechanism by which cationic angiogenic factors and ECM interact to regulate capillary endothelial cell growth depends upon their ability to modulate cell and nuclear form.

MATERIALS AND METHODS

Cell Culture. BCE cells were isolated from bovine adrenal glands as previously described (11) and passaged on gelatin-coated dishes in Dulbecco’s modified Eagle’s Medium (DMEM) supplemented with 10% calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 U/ml streptomycin (all from Gibco, Grand Island, ME), and 5 μl/ml retinal extract (14).

Matrix-Coating Procedures. Laminin, and types III, IV & V collagens were purified as previously described (33,35). Fibronectin (Cooper, Malvern, PA) and gelatin (Gibco; the same as used for cell passaging) were also used in these studies. All ECM components were diluted to 1 μg/ml in 0.1 M carbonate buffer, pH 9.4, plated at 100 μl (100 ng) per well of bacteriological plastic 96 well plates (Immunolon II; Dynatech Laboratories, Inc., Alexandria, VA), and allowed to adsorb by incubating overnight at 4°C. Amounts of matrix components bound were found to be in the range of 1 to 50 ng/cm² as determined using a published quantitative ELISA inhibition assay (36). These coating densities correspond to coverage of approximately 5 to 55% of the culture surface area (calculations were made based upon size and mass estimates that were determined by scanning transmission electron microscopy). Dishes prepared in this fashion supported optimal BCE cell attachment in the absence of serum.

Endothelial Growth Factors. HDGF was kindly supplied by Dr. M. Klagsbrun and Robert Sullivan (The Children’s Hospital) who purified this endothelial mitogen from human SK hepatoma cells using a combination of ion-exchange, heparin-affinity, and reversed-phase high pressure liquid chromatography (29). FGF and EGF were obtained from Collaborative Research.

Experimental System. Newly confluent BCE cells were cultured for 2 days free of retinal extract in DMEM containing 2.5% calf serum. Endothelial cell layers were dissociated into single cells with tryspin-EDTA (Gibco) and transferred into DMEM containing 1% bovine serum albumin (Fraction V; Sigma, St. Louis, MO). To study the effects of growth factor stimulation, cell suspensions were supplemented with saturating amounts of HDGF (2 ng/ml), FGF (100 ng/ml), or EGF (100 ng/ml), plated at 1 × 10⁴ cells/well on ECM-coated substrata and cultured at 37°C. Similar results were obtained with cells plated at lower density.

DNA Synthesis Assay. 3H-methyl thymidine (New England Nuclear, Boston, MA) was added to BCE culture media after 1 hr of culture (final concentration, 5 μCi/ml). Incorporation of radiolabel was terminated after 18 hr by addition of 100 fold excess of non-radioactive thymidine. Attached cells were washed with phosphate-buffered saline, rinsed with methanol, and DNA was precipitated on the dish with cold trichloracetic acid. Acid-precipitates were dissolved in 0.3 N NaOH and counted in a Beckman LS 3801 scintillation counter. In order to analyze DNA synthesis on a per cell basis, the number of cells in parallel wells was quantitated using a published assay based on cellular acid phosphatase (6).

Morphological Studies. To analyze alterations of cell shape, BCE cells were fixed in 2% glutaraldehyde and stained in 1% toluidine blue. Cells were photographed within 96 well plates using a Nikon Diaphot inverted...

FIG. 1. Regulation of Capillary Endothelial Cell DNA synthesis by ECM and HDGF. Incorporation of 3H-thymidine by BCE cells was measured after 18 hrs on different matrix-coated dishes in serum-free medium alone (stippled bars) or in medium supplemented with HDGF (black bars; 2 ng/ml) IV, type IV collagen; GEL, gelatin; III, type III collagen; V, type V collagen; FN, fibronectin; LM, laminin. Data presented are the result of three separate experiments. Error bars indicate standard error of the mean.