A-14 EFFECTS OF COLLAGEN MATRIX ON GROWTH AND DIFFERENTIATION OF CULTURED SMOOTH MUSCLE CELLS

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While vascular smooth muscle cells are thought to interact with extracellular matrices including various phenotypes of collagens, the precise collagen-cell interaction remains obscure. We examined the influence of type I and III collagens on the proliferation and differentiation of vascular smooth muscle cells in vitro.

MATERIALS AND METHODS

Cultured smooth muscle cells(7-12 PDL) derived from explants of the porcine abdominal aortic media were used for the experiments. 1X10^5 cells were seeded on plastic(plastic group), 0.2% type I(group I) and type III(group III) collagen gel substrata, respectively, and maintained in DME-12.5mM HEPES(pH 7.4) containing 10% FCS. After 1,3,5,7,9 days in culture, the cells were observed under a phase contrast microscope and then harvested by incubation with 0.05% trypsin-0.53mM EDTA in the case of the plastic group or 0.2% collagenase-1mM CaCl_2 in the case of groups I and III for cell counting. The incorporation of ^3H-thymidine into DNA was measured by pulse labeling for 2 hours on the 1st, 3rd and 5th days of culture. After 3,6,12 and 24 hours in culture, the cells were stained with NBD-phallacidin(1.6X10^-6 M), a specific probe for F-actin, and viewed through a fluorescence microscope. The frequency of cells with distinct actin filaments in the cytoplasm was compared in the glass and collagen I and III groups.
RESULTS AND DISCUSSION

The number of cells in the plastic group was much greater than that in groups I and III during the culture period. However, the difference between plastic and collagen groups decreased as the culture proceeded. Cells spread on collagen gels had a longer doubling time and incorporated less $^3$H-thymidine on the 1st day of culture than did cells in the plastic group. But the former showed a shorter doubling time and greater incorporation of $^3$H-thymidine on the 3rd and 5th days than the latter. Cells on collagen gels were more elongated than were those on plastic, and showed a "hills and valleys" arrangement from the 1st day of culture in group III (Fig.1). There was no formation of actin filaments during the first 3 hours of planting on any substrate. The majority of cells in all groups showed the formation of actin filaments after 12 hours, but the mean value for the frequency of cells forming actin filaments was higher in the glass group than in groups I and III. Cells on glass cover slips had filaments running in various directions within the cytoplasm. In contrast to this, cells on collagen gels had a large number of actin filaments travelling parallel to the direction of the major axis of their cytoplasm (Fig.2). Therefore, culture smooth muscle cells grown on collagen gels showed a suppression of the rate of proliferation and enhancement of differentiation in the early stages of culture.

Fig.1 Group III cells (1st day)  
Fig.2 Actin filaments in group I cells