ANALYSIS OF THE INTERACTION OF ADHESION AND GROWTH FACTOR INITIATED SIGNAL TRANSDUCTION DURING ANGIOGENESIS

Kamaljit K. Seehra*, Peter E. Shaw, Lopa Leach and Peter Jones

Department of Biochemistry, School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH
*mbxks@nottingham.ac.uk

INTRODUCTION. During angiogenesis, endothelial cells, like many other adherent cells require synergistic signals generated from integrin-mediated contact with surrounding extracellular matrices and from growth factors, to promote cell cycle progression, migration, capillary cord formation, tube formation and to suppress apoptosis.

Matrigel, a commonly used multimolecular matrix in vitro model of angiogenesis, contains an undefined range and quantity of growth factors providing a multifactorial nature to the system. To limit the number of signal transduction pathways activated we have used collagen type I as the extracellular matrix with vascular endothelial growth factor (VEGF), a potent pro-angiogenic factor within the angiogenesis model. Signal transduction pathways activated by VEGF are relatively well characterised, those involving the collagen type I integrins are less understood, although the α1β1 and α2β1 integrins have been demonstrated to play a key role (1).

METHOD. In vitro Angiogenesis assays consisting of matrigel and collagen type I were similar to those previously described (2,3). Assays were set up both in 2- and 3-dimensions and visualised under a standard light microscope.

RESULTS. It was found that a human microvascular cell line (HMEC-1) when cultured on collagen type I in contrast to; when cultured on matrigel (Fig. 1a) and behaviour shown by human umbilical vein endothelial cells (HUVEC), were unable to form capillary cord-like structures, although remaining viable upto 72hrs. Stimulation with VEGF (20ng/ml) failed to induce cord formation, although HMEC-1 were able to show a directional migration response. Furthermore, the use of a translation inhibitor, cycloheximide (5µg/ml), upon matrigel appeared to have no inhibitory effect on cord formations (Fig. 1b). Cytotoxic effects were observed at concentrations of 10µg/ml (Fig. 1c). The HMEC-1 integrin expression profile shows the presence of the α1β1 and α2β1 integrins whereas only the α1β1 is present on HUVEC (4). Function
blocking of the α1β1 in HMEC-1 with an antibody had no induced effect on collagen type I.

Fig. 1. Cycloheximide effect on capillary cord-like formations.

DISCUSSION. The data shows evidence of cord formation occurring in the absence of de novo protein synthesis, implying HMEC-1 have all the molecular elements required for this process.

Although not able to form capillary cords HMEC-1 are still able to migrate in response to VEGF, indicating that HMEC-1 migration and cord formation are regulated by at least two distinct signaling pathways and that cell migration per se is not sufficient for cord formation.

The data also shows α1β1 does not have an inhibitory effect upon cord formation suggesting that the collagen type I integrin signals are insufficient for cord formation and that signalling through other integrin heterodimer families are required for HMEC-1.

REFERENCES