BASEMENT MEMBRANE LAMININ-DERIVED PEPTIDE SIKVAV PROMOTES ANGIOGENESIS AND TUMOR GROWTH

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INTRODUCTION

The basement membrane is a thin extracellular matrix which underlies endothelial cells in vessels and forms a barrier to the passage of macromolecules and cells (Martin et al, 1988). Basement membranes also provide structural support and are very biologically active (Kleinman et al, 1987; Beck et al, 1990). The major and constant components of basement membranes include laminin, collagen IV, entactin, heparan sulfate proteoglycan and various growth factors (Martin et al, 1988; Vukicevic et al, 1992). These components interact with each other to form a highly elastic and organized structure.

Basement membranes are very biologically active with the response dependent on the specific cell type (Kleinman et al, 1993). Such activity has been demonstrated using either specific antibodies, isolated matrices such as the lens, or a sarcoma tumor extract reconstituted in vitro. The most direct results have been obtained using Matrigel, an extract of the Engelbreth-Holm-Swarm (EHS) tumor which can be coated on culture dishes (Kleinman et al, 1986). This extract contains the major basement components and various growth factors including EGF, bFGF, TGFβ, PDGF, and insulin-like growth factor 1 (Vukicevic et al, 1992). This extract is a liquid at 4°C and gels at 37°C. It can be used as a culture substratum or cells can be resuspended in the cold liquid before the gel is formed. In vitro, Matrigel promotes epithelial and endothelial cell differentiation and a greatly reduced proliferative rate is often observed with normal cells (Kubota et al, 1988). Cells differentiate in a highly specific manner. For example, salivary gland cells form gland-like structures (Kibbey et al, 1992b) while oviduct cells form tubes with secretory villi directed toward the lumen (Joshi, 1991). In addition, sea urchin micromeres differentiate and form spicules (Benson and Chuppa, 1990) and the entire life cycle of the avian malaria parasite Plasmodium is completed on Matrigel (Warburg and Miller, 1992). Furthermore, endothelial cells form capillary-like structures with a lumen (Kubota et al, 1988). Matrigel has also been used in vivo to facilitate tumor growth (Kibbey et al, 1992a), assay for angiogenesis (Passaniti et al, 1993), increase neural graft survival (Haber et al, 1988), and stimulate epithelialization of intestinal defects (Thompson, 1990).

BIOLOGICAL ACTIVITIES OF LAMININ

Laminin, a major glycoprotein in basement membranes, is one of several very active species in this matrix. Laminin, as isolated from the EHS tumor, has been found to promote cell adhesion, migration, growth, differentiation, neurite outgrowth, tumor growth and metastases, and increased activity of tyrosine hydroxylase and collagenase IV (Kleinman et al, 1993b). Laminin is composed of three chains designated A(Mr = 400,000), B1(Mr = 210,000), and B2(Mr = 200,000) (Figure 1).
Several homologues of laminin have been described which are also biologically active. When cells adhere to laminin, they generally undergo a distinct and cell type specific morphogenesis unlike fibronectin-mediated adhesion where cell spreading is most commonly observed. On laminin, for example, Sertoli cells become more columnar (Suarez-Quian et al., 1985) whereas Schwann cells become more elongated (McGarvey et al., 1984). Laminin is of particular interest because of its ability to promote neurite outgrowth with many neuronal cells and to facilitate nerve regeneration in vivo. Laminin has also been shown to promote the malignant phenotype. Laminin-adherent tumor cells in vitro are more malignant when injected in vivo (Terranova et al., 1984; Jun et al., 1993). The level of laminin 32/67Kd receptors correlates with malignancy (Wewer et al., 1983) and intravenous coinjection of laminin with melanoma cells results in increased numbers of colonies on the surface of the lungs (Barsky et al., 1984). Furthermore, antibodies to laminin decrease lung colonization. Laminin also increases the activity of collagenase IV, an enzyme responsible in part for tumor spread (Turpeeniemi-Hujanen et al., 1986). These studies demonstrate important functions for laminin in development and in disease. It is likely that specific clinical formulations of laminin may be used in facilitating tissue repair and in inhibiting certain disease processes.

Various active domains of laminin have been described at the synthetic peptide level (Beck et al., 1990; Kleinman et al., 1993b). These laminin-derived synthetic peptides have different biological functions. The most studied peptides include YIGSR (residues 929-933 on the B1 chain), RGD (residues 1118-1128 on the A chain), and SIKVAV (residues 2099-2105 on the A chain). All three peptides promote cell adhesion but are cell type specific. For example, RGD is most active with endothelial cells (Grant et al., 1989) whereas SIKVAV is most active with neuronal cells (Tashiro et al., 1989). Interestingly YIGSR inhibits angiogenesis in vitro and in vivo (Sakamoto et al., 1990) whereas SIKVAV has the opposite activity (Kibbey et al., 1992a; Grant et al., 1992). It is likely that all of the active sites on laminin are not available (active) simultaneously due to both conformational changes and steric blocking by interacting macromolecules.

The SIKVAV peptide from the laminin A chain is unusually active with a variety of cells (Table I). This peptide sticks very well to plastic and promotes the adhesion of many cells. Cells migrate well to this peptide in Boyden chamber assays and appear more migratory on surfaces coated with the peptide (Tashiro et al., 1989; Grant et al., 1992). This peptide has been found to promote cell growth but it is probably not the main growth promoting site on laminin.