Distribution of Fibronectin and Laminin in Human Liver Tumors

Miklós Szendrői and Károly Lapis
First Institute of Pathology and Experimental Cancer Research, Semmelweis University Medical School, Budapest, Hungary

Summary. Two extracellular matrix and basement-membrane components, fibronectin (Fn) and laminin, were studied by the indirect immunoperoxidase technique in ten primary human liver cancers. Similar distributions of both Fn and laminin were detected in the well differentiated hepatocellular carcinomas with trabecular and tubular pattern. Two moderately differentiated hepatomas contained Fn only. Neither Fn nor laminin were present, however, in the parenchyma of one poorly differentiated hepatoma. In three cases of cholangiocarcinoma, laminin surrounded the tumorous ducts, while Fn appeared mainly in the reactive connective tissue stroma.

The present findings indicate that bile-duct cancers synthesize laminin, and not Fn while differentiated hepatocellular carcinomas produce both Fn and laminin in vivo. The presence of Fn even in moderately differentiated types of liver cancer is in contrast to the findings for carcinomas developing from other organs and it may serve as a marker for primary hepatocellular carcinomas in the differential diagnosis.

Key words: Fibronectin - Laminin - Liver cancer

Introduction
Both fibronectin (Fn) and laminin are high-molecular-weight noncollagenous glycoproteins of the extracellular tissue matrix. The former has previously been shown to be present at different cell surfaces, in the loose connective tissue, in blood, and other body fluids (Ruoslahti et al. 1981). It interacts with many macromolecules, such as collagens, glycosaminoglycans, actin filaments, and cell-surface receptors, and it is associated with basement membranes in the tissues (Engvall et al. 1978; Stenman and Vaheri 1978). Mesenchymal cells and several types of epithelial cells have been found to produce Fn in culture (Smith et al. 1979). These cells, however, lose or reduce the cell-surface Fn during their malignant transformation (Vaheri and Ruoslahti, 1974).

Laminin, a glycoprotein component of basement membranes, has recently been isolated from a mouse tumor and from human placental and renal basement membranes (Risteli and Timpl 1981; Timpl et al. 1979). It consists of two disulfide-linked and glycosylated polypeptide chains with molecular weights of about 200,000 and 400,000. Laminin shares some common features with Fn (affinity for glycosaminoglycans, heparin, and heparan sulfate), but they are biochemically and immunochemically distinct from each other (Chung et al. 1979). The distribution patterns of the two glycoproteins are similar but laminin is less abundant in the connective tissue (Hahn et al. 1980). The role of laminin is only slightly understood but in vitro studies indicate that laminin may accelerate the attachment of the epithelial cells to the underlying basement membranes (Carlsson et al. 1981).

Material and Methods
Tissue specimens of ten primary liver carcinomas were obtained from biopsy or fresh autopsy material. The samples were quick frozen, and the 4-6 μm-thick cryostat sections were fixed for 30 min in 6% formal PBS solution containing 4% sucrose. Indirect immunoperoxidase technique was performed with specific rabbit antisera to human Fn and human laminin (both antisera kindly provided by Prof. Vaheri, Helsinki). The first antiserum was diluted 1:40 and the second 1:100, both in PBS. Peroxidase-labelled goat antiserum to rabbit immunoglobulins (Institut Pasteur, France) was used as the second antiserum (1:80 and 1:150 in PBS).Sections were counterstained with 1% hematoxylin. Controls included nonimmunized rabbit serum as first antibody or peroxidase labelled goat IgG to rabbit Ig alone (1:20 in PBS). All controls were negative. Besides immu-
noperoxidase staining, cryostat sections were also stained with PAS and picrosirius red F3BA for glycogen and connective tissue.

Results

From the ten primary liver tumors collected, six proved to be hepatocellular carcinomas and three were cholangiocarcinomas. In one case, the rare diagnosis of hepatocholangiocarcinoma could be made. The sex distribution was: six male and four female. In three of the six hepatocellular carcinomas studied the microscopic findings showed a fair degree of differentiation. Two of these tumors and the hepatocholangiocarcinoma occurred in young patients (median age: 21.6 years) and in the absence of a preexisting cirrhosis. All other tumors arose in livers that were the seat of advanced cirrhosis (median age: 67.8 years).

The three well differentiated hepatocellular carcinomas with tubular and trabecular structures and the hepatocholangiocellular carcinoma contained Fn and laminin in a similar distribution (Fig. 1a, b). Fibronectin was present not only in the reactive connective tissue stroma of the hepatomas, but also in irregular fibrillar and poollike form in the extracellular spaces of the parenchyma and on the surface and in the cytoplasm of the tumors.