Expression of laminin, type IV collagen and fibronectin molecules is related to embryonal skin and epidermal appendage morphogenesis

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Summary

Basement membrane zones are specialized sheets – like arrangements of extracellular matrix proteins and glycosaminoglycans, and act as an interface between parenchymal cells and support tissue. They separate epithelium, endothelium, muscle cells and Schwann cells from adjacent connective tissue stroma, and also from a limiting membrane in the central nervous system. They are involved in several cellular and biological processes, including adhesion, migration and cellular differentiation.

Basement membranes have five major components: collagen type IV, laminin, heparan sulfate, entactin, and fibronectin. In addition, there are numerous minor and poorly characterized protein and glycosaminoglycan components.

The various components of the basement membranes of the skin (collagen type IV, proteoglycans - heparan sulfate, laminin, entactin and fibronectin) are products of the epithelial (epidermal) cells.

We studied immunohistochemically the origin, the first appearance and distribution of the adhesive extracellular glycoprotein laminin and the fibrillar proteins of the extracellular matrix collagen type IV and fibronectin in the basement membranes of fetal human skin between 12 to 21 weeks of gestational age. Additionally, we studied the expression of vimentin in the extracellular matrix of the epithelial/mesenchyme junction of the skin.

This study demonstrates clearly that the expression of the antigens laminin, collagen type IV and fibronectin starts in the germinative epithelial cells of the skin at the bulbs of the hair follicles (12th week for fibronectin and 19th week for laminin and collagen type IV), and migrating progressively involves the epithelial epidermal cells of the covering skin, as well as, the basement membrane at the dermal-epidermal junction in that region (between 20 to 21 weeks of gestational age).

Key words: Laminin; Collagen type IV; Fibronectin; Fetal skin.

Introduction

Embryology. Skin is developed from the surface ectoderm and its underlying mesenchyme. Surface ectoderm gives rise to the keratinizing general surface epidermis and its appendages, the pilosebaceous units, sudoriferous glands and nail units.

Epidermal/mesenchymal interactions involving mutual inductive mechanisms are important during development and postnatally [1]. They occur at the interface between the two, the basement membrane zone (BMZ), the development of which has been widely studied in morphological, biochemical, and immunological terms [2-4]. Hemidesmosomes begin to appear at 8 weeks as stratification starts, and anchoring fibrils at 9-10 weeks. By the end of the third month the basic morphology of the interfollicular BMZ is essentially similar to that postnatally.

The basement membrane provides a physical supporting substrate and attachment for the developing epidermis, and is thought to be selectively permeable to macromolecules and soluble factors regulating epidermal-dermal morphogenetic interactions. These have mainly been studied in other species, and in vitro [3, 5], but it is likely that the general principles also apply in human development.

Biochemically, basement membranes (BM) are formed by heterogeneous molecules, the laminin and type IV collagen being ubiquitous components, but differences in BM composition exist among different tissues [6, 7].

The distribution of basement membrane components including, laminin, fibronectin and type IV collagen in various epithelial and non-epithelial cells has been widely studied [6, 7]. Nevertheless, as far as we know, no data have been published on the first presence and localization of basement membrane components of fetal human skin between 12 and 21 weeks of gestational age. Furthermore, the origin and the distribution of these molecules in the developing epidermal cells and mesenchymal cells of the dermis were studied in parallel.

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Fibrillar proteins of the extracellular matrix:

I. *Collagen type IV.* Collagen type IV is the major collagen and represents one of approximately 16 types of collagen currently characterized in the body. Type IV collagen does not form fibrils, as do many of the other collagens. It consists of short filaments that are thought to provide structural integrity to the basement membrane. Moreover, it has a much higher content of hydroxyproline, hydroxylysine, and carbohydrate side chains than do other collagens.

II. *Fibronectin.* Fibronectin is a multi-functional glycoprotein and exists in three main forms. These are: a circulating plasma protein, a protein that transiently attaches to the surface of many cells, insoluble fibrils forming part of the extracellular matrix, when fibronectin dimmers cross-link to each other by disulphide bonds. The functional importance of fibronectin stems from its ability to adhere to several different tissue components because it possesses sites binding collagen and heparin, as well as cell adhesion molecules. Fibronectin is recognized by fibronectin receptor proteins in cell membranes (integrins), allowing cell adhesion to the extracellular matrix.

Extracellular structural glycoprotein: Laminin.

Laminin is a sulphated glycoprotein and is a major component of basement membranes. It is produced by most epithelial and endothelial cells, and is a cross-shaped molecule with binding sites for specific cell receptors (integrins), heparan sulfate, type IV collagen, and entactin. The multiple binding ligands for laminin make it a major extracellular link molecule between cells and extracellular matrix.

Intermediate filament: Vimentin.

Vimentin filaments are characteristic of cells of mesenchymal origin and of embryonic or undifferentiated cells. Vimentin is a single protein (MW 56,000-58,000) and may copolymerize with desmin or glial fibrillary acidic protein.

Materials and Methods

Source of tissues

Specimens of skin from the sculp were obtained from autopsies after legal abortion. The embryos were between 10 and 20 weeks of gestational age. Autopsies were performed a few days after the intrauterine death. Samples of skin were fixed with 10% formaldehyde and processed for routine embedding in paraffin. Paraffin-embedded blocks were cut in serial sections 5 m to 7 m thick and stained with:

1. Haematoxylin-eosin (routine examination) in order to demonstrate the general histologic architecture of two main layers, the epidermis and dermis, and the variable third layer, the subcutis.
2. Periodic acid-Schiff (PAS) to demonstrate carbohydrates and carbohydrogenic macromolecules in the connective tissue.
3. Masson's trichrome to demonstrate the architecture of the muscle cells and collagenous matrix.
4. Verhoeff-van Giesson to demonstrate the architecture of the elastic fibers.
5. Immunohistochemical stains to demonstrate:

a) The adhesive extracellular structural glycoprotein, laminin;

b) The fibrillar protein of the extracellular matrix, collagen type IV and fibronectin;

c) The intermediate filamentous cytoskeletal protein, vimentin.

Results

Immunohistochemical reaction at 12 weeks of gestational age: Laminin and collagen type IV were negative in the basement membranes of the skin and in the epithlial cells of the epidermis and skin appendages. In contrast, fibronectin was identified in the extracellular matrix of the skin, including the basement membranes at the dermal-epidermal junction (Figure 1) and skin appendages. Furthermore, a positive reaction for fibronectin was seen in the cytoplasm of the germinative epithelial cells of the hair bulbs in the dermis (Figure 2). Vimentin staining showed a positive reaction in the cytoplasm of the mesenchymal cells in the papillary and reticular dermis (Figure 3).

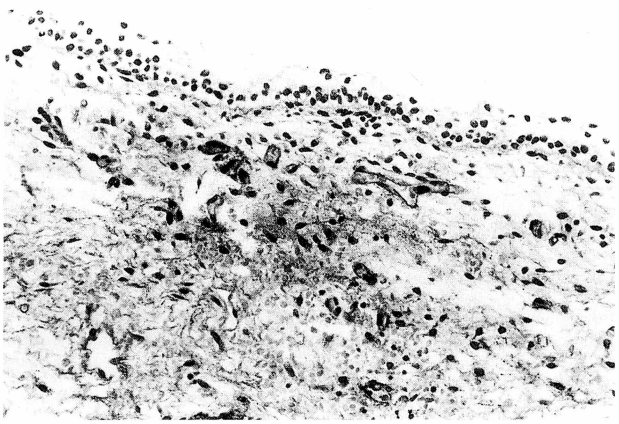

Figure 1. — Micrograph of the extracellular matrix and basement membrane of the fetal skin at the 12th week of gestational age which were immunostained for fibronectin (x100).

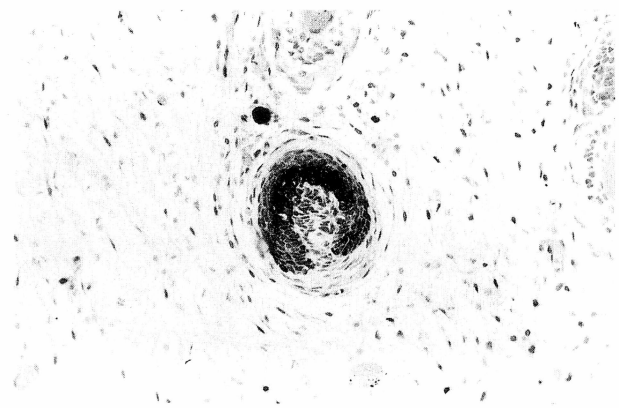

Figure 2. — Micrograph showing a positive immunohistochemical reaction for fibronectin in the cytoplasm of the germinative epithelial cells of the hairs bulbs in the dermis of fetal skin (12th week of gestational age) (x200).

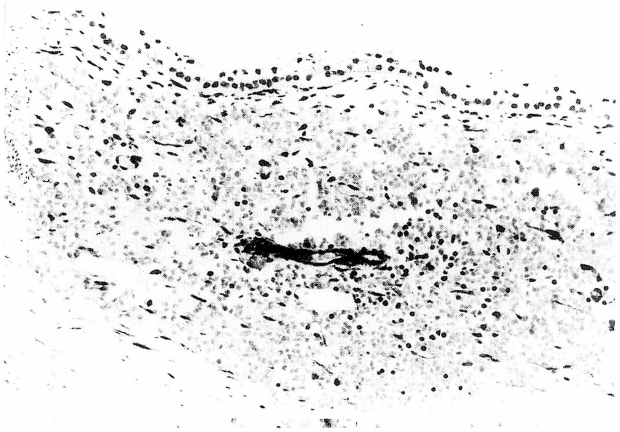

Figure 3. — Micrograph showing a positive immunohistochemical reaction for vimentin in the cytoplasm of the mesenchymal cells in the papillary and reticular dermis of the fetal skin (12th week of gestational age) (x200).

Immunohistochemical reaction at 19 weeks of gestational age: A positive reaction was shown for laminin and collagen type IV in the germinative epithelial cells of the hair bulbs in the dermis (Figure 4). No staining was seen for these antibodies in the basement membranes (dermal-epidermal junction, skin appendages. Fibronectin showed the same reaction as at the 12th week of gestational age.

Immunohistochemical reaction at 20 to 21 weeks of gestational age: The staining for laminin, collagen type IV and fibronectin antibodies showed at this time a strong positive reaction in the cytoplasm of the epithelial cells of the hair shafts and a weak positive reaction in the cytoplasm of the basal cells of the epidermis, especially in the region where the hairs open to the epidermal surface (Figure 5). Furthermore, in that region a weak positive reaction was seen in the basement membrane at the dermal-epidermal junction.

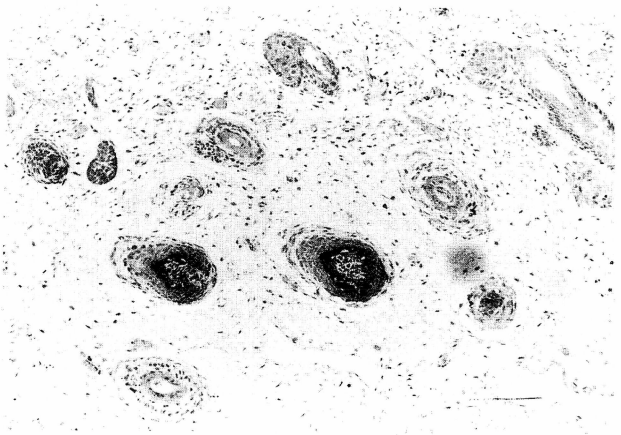

Figure 4. — A positive immunohistochemical reaction for laminin in the cytoplasm of the germinative epithelial cells of the hair bulbs in the fetal dermis (19th week of gestational age) (x200).

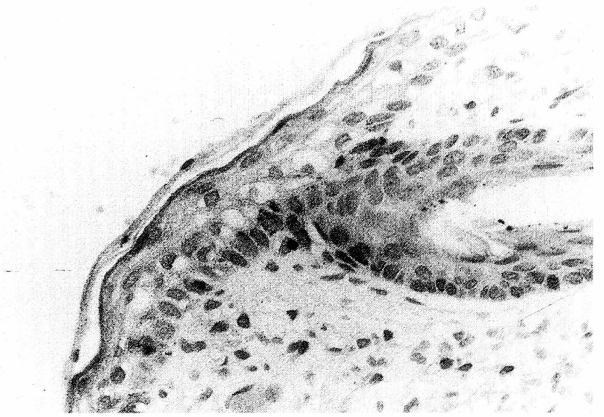

Figure 5. — Micrograph showing a strong positive immunohistochemical reaction for collagen type IV in the cytoplasm of the epithelial cells of the hair shafts, and a weak positive reaction in the cytoplasm of the basal cells of the epidermis, especially in the region where the hairs open to the epidermal surface. Note the weak positive immunoreaction in the basement membrane at the dermal-epidermal junction (20th to 21st week of gestational age) (x400).

Discussion

The present findings indicate that fibronectin at 12 weeks of gestational age was identified in the extracellular matrix of the skin (basement membranes at the dermal-epidermal junction and skin appendages), and in the cytoplasm of the germinative epithelial cells of the hair bulbs in the dermis. Vimentin reaction was positive in the spindle-mesenchymal cells of the dermis.

At 19 weeks of gestation germinative epithelial cells of the hair bulbs in the dermis were seen, whilst no staining was seen for these antibodies in the basement membranes at the dermal-epidermal junction and skin appendages. At 20 to 21 weeks of gestational age a weak positive reaction for laminin, collagen type IV and fibronectin was seen in the cytoplasm of the basal cells of the epidermis in the region where the hair opens to the epidermal surface, as well as in the basement membrane in that region.

These results lead to the conclusion that the expression of the antigens, laminin, collagen type IV and fibronectin starts in the germinative epithelial cells of the skin at the bulbs of the hair follicles (12th week for fibronectin and 19th week for laminin and collagen type IV) and progressively migrating along the structure of the shaft involves that of the covering skin (between 20 to 21 weeks of gestational age), as well as the basement membrane at the dermal-epidermal junction in that region (between 20 to 21 weeks of gestational age).

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