Expression of the intermediate filament vimentin and fibrillar proteins of the extracellular matrix related to embryonal heart development

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Summary

During organogenesis, the heart is one the first organs to develop and the earliest organ to function. The early appearance of cardiac activity in the tubular hearts of chick and rat embryos was noted many years ago [1, 2]. It arises from two plates of the splanchnic mesoderm which fuse to form a single tubular structure composed of endocardial and myocardial cells and, between them, the extracellular cardiac matrix. There is considerable variation in the formation of the extracellular matrix in the various regions of the heart during development.

The endocardial lining cells of the vertebrate embryos show a regional specificity that remains an unexplained phenomenon in cardiac morphogenesis. The great majority of the endocardial lining cells remain epithlial. However, a restricted population of endothelial cells, lining the atrioventricular (AV) canal and the reputed proximal outflow tract (OT), transforms into mesenchyme; the latter being the reputed progenitor of the valves and membranous septa.

The purpose of this study was to investigate the extracellular cardiac matrix of the human fetal heart in different regions and in various stages of development, and also the heterogeneity of the endocardial cell lining, in connection with the endothelial cells of other cardiac vessels.

Identification of the mesenchymal cells/extracellular matrix was confirmed by immunohistochemical techniques using the following monoclonal antibodies: actin, desmin, vimentin, collagen IV and fibronectin.

The present results provide evidence that the extracellular matrix of the heart is of mesodermal origin but at the level of the valves the mesenchyme is derived from the endothelial lining cells rather than the primitive mesenchyme.

Key words: Laminin; Collagen type IV; Fibronectin; Vimentin; Fetal heart.

Introduction

The entire cardiovascular system (the heart, blood vessels, and blood cells) originate from the mesodermal germ layer. Although initially paired, by the 22nd day of development two tubes form a single, slightly bent tube, consisting of an inner endocardial tube and a surrounding epimyocardial mantle [3]. During the 4th to the 7th weeks, the tubular structure of the heart develops into a multichambered, specialized organ. This transformation is initiated by a regionally specific transformation of the endocardial cells into migrating and proliferating mesenchymal cells [4]. These mesenchymal cells colonize the cardiac jelly in the outflow tract and at the atrioventricular junction to form both the outflow tract and the atrioventricular endocardial cushions [5]. These transient endocardial cushions are involved in the formation of the connective tissue of the interventricular septum, the semilunar and atrioventricular valves of the heart. Furthermore, the heart originates to a lesser extent from neural crest cells [6-8].

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The extracellular matrix of the heart, historically termed cardiac jelly, gelatinoreticulum, myoepicardial reticulum and more recently the myocardial basement membrame [9], is composed of, among other elements, hyaluronic acid, hyaluronidase and fibronectin.

Intermediate filaments:

1) *Actin.* Actin is an important component of the cytoskeleton and accounts for about 5% of the total protein in most cell types. It is a globular protein (G-actin), which polymerizes to form filaments (F-actin) with all the subunits facing in one direction (polar filaments). There are six molecular variants (isoforms) of actin, which have specific distributions in different cell types, for example isoforms restricted to smooth muscle or skeletal muscle.

2) *Desmin (skeleton).* Desmin is found in smooth muscle and in the Z disks of skeletal and cardiac muscle (MW 53,000 - 55,000).

3) *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.

Fibrillar Proteins of the extracellular matrix:

1) *Collagen type IV.* Collagen type IV, which makes up a very large proportion (about 30%) of all the proteins of the body, was formerly thought to be a single protein with an amino-acid composition that had been highly conserved in the course of evolution, but improved methods of analysis have led to the discovery of differences in the collagen extracted from various tissues in the body. Collagen is now regarded as a family of closely related, but genetically distinct proteins that share certain features of molecular organization but have a-chains that differ in their amino-acid composition and sequence. Collagens are classified using roman numerals to reflect the chronological order of their discovery. At least 14 types are now generically characterized and others are being investigated. Type IV collagen is a specialized form largely restricted to the basal lamina of epithelia. Together with laminin and heparan sulfate proteglycan, it forms a close meshwork of fine filaments that is the physical support of epithelia and a selective barrier for macromolecules.

2) *Fibronectin.* Fibronectin is a multi-functional glycoprotein and exists in three main forms. These are: a) a circulating plasma protein, b) a protein that transiently attaches to the surface of many cells, c) insoluble fibrils forming part of the extracellular matrix, when fibronectin dimers cross-link to each other by disulphide bonds. The functional importance of fibronectin stems from its possession of sites which bind collagen and heparin, as well as cell adhesion molebranes (integrins), allowing cell adhesion to the extracellular matrix.

Materials and Methods

Source of tissues

Specimens of human fetal heart were obtained from autopsies after legal abortion. The embryos were between 10 and 23 weeks of gestational age. Autopsies were performed a few days after the intrauterine death. Samples of cardiac tissue were fixed in 10% formaldehyde and processed routinely in paraffin wax. Tissue blocks were cut in serial sections 5 m to 7 m thick and stained immunohistochemically in order to demonstrate:

a) The intermediate filamentous cytoskeletal protein of the muscle cells, i.e actin and desmin.

b) The intermediate filamentous cytoskeletal protein of the fibroblast, i.e vimentin.

c) The fibrillar protein of the extracellular matrix, i. e., collagen type IV.

d) The extracellular matrix component, i.e., fibronectin.

Results

Expression of actin. In 10 to 12 week embryos actin reacted strongly with the endothelium and the muscle coat of all blood vessels, and with the endocardial cells lining both atrial and ventricular chambers. There was a strong positive staining in the intercellular substance of the atrioventricular and semilunar valves.

Expression of desmin. Desmin was positive in only one case, and was confined to the endocardial lining cells in the atria, atrioventricular valves and pericardium.

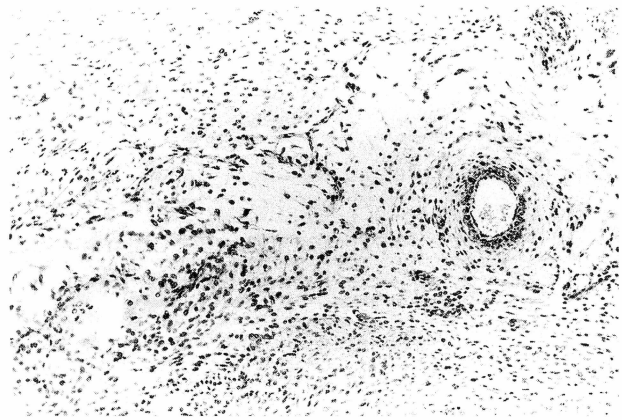

Figure 1. — Micrograph showing a strong positive immunohistochemical reaction for vimentin in the cytoplasm of the spindle cells in the middle layer of the blood vessels around the semilunar valves (fetal heart at 10th week of gestational age) (x100).

Expression of vimentin. Vimentin showed a strong positive reaction at the level of the four cardiac valves (central fibrous body) and middle layer of the blood vessels around the semilunar valves of the embryos at 10 weeks (Figure 1). Specimens of subsequent stages of development showed a variable positivity for vimentin at the middle and innermost layer of the atria, ventricles and atrioventricular valves.

Expression of collagen type IV. The immunohistochemical analysis for the detection of collagen type IV showed negative staining.

Expression of fibronectin. At 10 weeks' gestational age, fibronectin showed a strong positive reaction in all three layers of the endocardium and adjacent strong positive reaction in all three layers of the endocardium and adjacent muscle tissue of the atrioventricular and semilunar valves (Figure 2). Cardiac specimens of subsequent stages of development showed a decreased amount of fibronectin. In the latter cases, this fibrillar protein was confined mainly in the endocardium and adjacent muscle and fibrocollagenous tissue of the valves, as well as, at

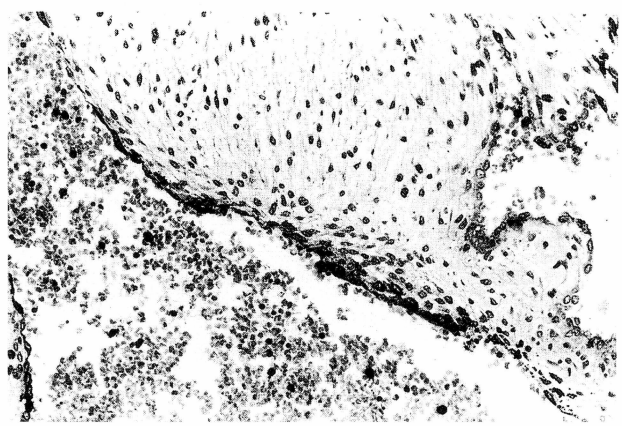

Figure 2. — Micrograph showing a strong positive immunohistochemical reaction for fibronectin in all three layers of the endocardium and adjacent muscle tissue of the atrioventricular valves (fetal heart at 10th week of gestational age) (x100).

the walls of blood vessels - especially those around the semilunar valves. Furthermore, fibronectin showed a positive reaction to the central fibrous body of the heart, middle layer and adventitia of the blood vessels around the semilunar valves and pericardium.

Discussion

The purpose of the present study was to investigate immunohistochemically the extracellular matrix in different cardiac regions and in various periods of development (10 to 23 weeks of gestational age), as well as, the diversity of the endothelial lining cells of the cardiac chambers and vessels.

These results led to the conclusions:

The extracellular matrix of the heart at the level of the atrioventricular and semilunar valves is different from that in the atria, ventricles and blood vessels, as shown by the immunoreactivity to fibronectin.

There is a regional specificity of endothelial cells lining the atrioventricular and semilunar valves, as shown by the increase of the substrate adhesive molecule fibronectin and absence of collagen type IV in these cells. Inductive factors originating probably from the myocardial cells transform the endocardial cells lining the atriventricular canal and proximal outflow tract into mesenchyme (cardiac mesenchyme), while the endocardial cells in other regions of the heart, i.e., in the ventricles, remain epithelial [9]. This myocardial activation is expressed by the endocardial cells with increased fibronectin in 10 to 12 week embryos.

Fibronectin is a prominent component of the atrioventricular and semilunar valves.

The extracellular matrix of the heart is of mesodermal origin but at the level of the valves the mesenchyme is derived from the endothlial lining cells rather than the primitive mesenchyme.

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