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REVIEW ARTICLE

Light Microscopic Morphological Characteristics and Data on the Ultrastructure of the Cardiomyocytes.

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ABSTRACT

Many questions regarding the morphology of the cardiovascular system are yet to be answered. In particular, elucidating the core principles of the architectonics of the myocardium is of great importance for the understanding of the exact mechanisms of the cardiac functions and the pathogenic processes which constitute a prerequisite for cardiovascular diseases. A number of contemporary studies reveal the importance of the myocardium in almost every disease – either as a primary pathophysiological unit or as the target of the pathological damage. It has to be stated that the myocardium has a remarkable diagnostic and therapeutic potential. It is comprised of various types of cells – contractile cardiomyocytes of the atria and ventricles, cells of the sinoatrial node and Purkinje fibres, the latter two being part of the conducting system of the heart. The ultrastructural components of these cells include the various structures which ensure cellular contact and communication, the specialised structures of the cellular and the sarcoplasmic membrane and the different elements of the complex cytoskeleton. Furthermore, the orientation of the cardiomyocytes plays a key role not only for the mechanical contraction but also in the electric conduction and the energy metabolism of the cardiac muscle. Studies on the size, alignment and specific characteristics of the cardiomyocytes have the potential to provide a morphological base for the diagnostics of various cardiac pathologies.

Keywords: *Morphology, myocardium, structure, cardiomyocytes.*

INTRODUCTION

The normal cardiac function depends on the coordinated activity of a few main types of cells in the myocardium: cells of the sinoatrial node, which are known for the initiation of the cardiac rhythm; Purkinje cells, a main component of the specialized conduction system; operating cells of the ventricles and atria (contractile cardiomyocytes), which execute the mechanic work. Regardless of the fact that the roles of the impulse conducting and contractile cells are very different, many of their ultrastructural characteristics are identical. In addition to the single nucleus, each muscle fibre contains chains of contractile units (sarcomeres), which have mitochondria that generate the energy for the operation of the cell; also, every cell has a sarcoplasmic (smooth endoplasmic) reticulum, which plays a main role in the relaxation of the cell.[1]

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LIGHT MICROSCOPIC MORPHOLOGICAL CHARACTERISTICS OF THE CARDIOMYOCYTES

James et al. describes a population of cells in the sinus node, as well as in the atrioventricular node, which consists of relatively primitive cells.[2,3] They are round or elliptic, do not branch and are characterized by a relative lack of electron-dense material in the cytoplasm. Except for the large, eccentrically placed

nucleus, they contain only an insignificant number of small mitochondria and scarce population of myofibrils with uneven distribution and length of barely a few sarcomeres. Due to the optically empty appearance of their sarcoplasm in comparison with the other cells of the heart, James calls them pale or P-cells. In most cases, the cell membranes rarely make contact with one another, in narrow zones; intercalated discs or other ultrastructural specialized junctions are not found between those cells.[1]

The contractile cardiomyocytes in the atria have an oval shape and do not ramify. Their diameter is approximately 6-8 µm and their length reaches up to 20-30 µm.[1] Tightly arranged chains of sarcomeres are found inside the cell. Among them, elliptical mitochondria with a complex system of folded membranes or cristae are located, on which the biochemical reactions connected with the production of energy take place. Unlike the cells of the ventricles, most of the atrial cells do not have a system of sarcolemmal invaginations that emerge from the cell surface and prolong into the cytoplasm associated with the myofibril (known as transversal tubular system or T-system). The lack of T-system decreases the overall surface of the membrane compared to the cells of the ventricles.[1]

The system of intercellular contacts in the atria is unique and different from the one in other parts of the myocardium. Atrial myocardium consists of bundles of 2 to 3 cells, situated very close to one another with their lateral surfaces. The intercellular space, in its widest part is just 0.2-0.3 µm. It is progressively narrowing, finally forming series of desmosomes and gap junctions between adjacent sarcolemmas – a short, almost linear and horizontally oriented intercalated disk, unique for the cardiomyocytes of the atria.[1] There are also contact zones between the cells resembling a standard intercalated disk found in the cardiomyocytes of the ventricles, being oriented perpendicularly to the long axis of the cell and having stair-like configuration. Contacts of this type are more rarely seen than the horizontal type. Intercellular spaces are rich in collagen, in which a network of capillaries and nerve fibres is present. This arrangement of intercellular contacts allows the impulse to pass from one cell to the other in both longitudinal and transverse direction.[1]

Ventricular cells are the biggest in the myocardium, with length of up to 100 µm. However, their mean diameter – 10-15 µm, although still larger than that of the atrial cells is smaller than that of Purkinje fibres. Furthermore, a large number of studies have demonstrated differences in some morphometric markers between the cardiomyocytes of the two ventricles. In addition, changes in these quantitative indices throughout different stages of the postnatal development have been shown in suitable experimental models.[4-6] As a whole, the organization of the tissue and of the intercellular contacts differs from the one in the atria. Muscle fibres in the ventricles are very closely located, forming cords of branching cells, which are more often connected via longitudinally oriented intercalated disks. Usually, the cardiomyocyte is branched and is connected to its adjacent cells via longitudinal intercalated discs. It is striated, and has one or two centrally located, slightly elongated, low density nuclei.[7] The cytoplasm of the myocyte is filled with alternating chains of sarcomeres and mitochondria.[1] At the level of the sarcomere, the T-tubule is closely associated with specialized extensions of the sarcoplasmic reticulum, called lateral cysterns, forming the so-called diades.[1] Another characteristic of the cardiomyocyte is the endoplasmic reticulum located in the perinuclear zone and its associated enzymatic systems, related to processes aimed at protection against different factors arising from the environment surrounding the cell, anti-hypertrophy functions and prevention of oxidative stress with advancing age.[8-10]

Purkinje fibres are specialized, fast conducting fibres of the ventricular myocardium.[11] Their diameter is 70-80 µm, making them the widest cells in the myocardium and is one of the reasons for the fast conduction. Purkinje fibres are abundant with linearly arranged sarcomeres, homogenously dispersed like those of ventricular contractile myocytes. The arrangement of myofibrils is interrupted at times by large, optically empty spaces of sarcoplasm, filled with glycogen granules, mitochondria and tubules of the sarcoplasmic reticulum.[12] Purkinje fibres do not possess a T-tubule system. Only sparse diades or triades are found in the transitional cells between Purkinje fibres and contractile cardiomyocytes. The membrane capacity of Purkinje cells is bigger than that of cardiomyocytes.[13] This can be explained by their large surface, provided by the intercalated disk, which is much more complex in Purkinje fibres. Conducting fibres are often arranged like a "Y" with the participation of three Purkinje cells. There are zones with mozaically arranged cells. Despite the lack of desmosomes and gap junctions, adjacent sarcolemmas are not more than 30-40 nm away. This type of cellular organization, as well as the complicated form of the intercalated disk are important for Purkinje cells' fast conduction.[1] [Table 1]

ULTRASTRUCTURE OF THE CARDIOMYOCYTES

The arrangement of the cardiomyocytes leads to a formation of a network or functional syncytium, which is the main structural unit of the myocardium. However, other cell types, such as fibroblasts, endothelium cells

and smooth muscle cells can be found in the myocardium.[14,15] The significance of the fibroblasts and the enzymes which they produce, particularly enzymes of the group of matrix metalloproteinases, is especially important. These enzymes have a number of essential biological properties. They participate in a variety of processes such as regeneration, migration and proliferation not only in the myocardium, but in other organs too, both under physiological and pathological conditions.[16-19] The connective tissue, which surrounds the cardiomyocytes includes three layers – epimysium, perimysium and endomysium. The normal extracellular matrix (cardiac gel) is produced by the cardiomyocytes and the fibroblasts in the myocardium. It is built of collagen type I, III and IV, laminin, fibronectin and proteoglycans. The change in the quantity of the collagen fibres of the connective tissue is the most significant marker of physiological processes such as aging of the myocardium, as well as pathological conditions associated with increased load and impaired function of the heart.[20-23]

The intercalated disc is the zone of contact between the adjacent cardiomyocytes in longitudinal direction. It consists of two parts: transversal part and lateral part. The intercalated disc shows certain differences of its morphology depending on the myocardial cell type (cells of the atrium, of the ventricle and of the conduction system of the heart).[24] In fact, the intercalated disc is a synaptic complex with a specific function – ensuring correct connection and communication between cardiomyocytes. Functionally, the disc consists of desmosomes or maculae adherentes, adherent junctions or fasciae adherentes and gap junctions. Desmosomes prevent cell division in conventional contractile activity. They are built from transmembrane proteins desmoglein-2 and desmocoline-2.[25] Adherent junctions serve as attachment sites for the actin filaments of the terminal sarcomeres.[26] The main membrane proteins composing the adherent junctions are N-cadherin and β1D-integrin, which probably serve as receptors for stretching.[27] Gap junctions or nexuses or maculae communicantes are important structures of the ionic transport between the adjacent cardiomyocytes and are built of connexins.[24] The main connexin in the myocardium of the ventricles is connexin-43, while connexin-40 and connexin-45 show differences in their quantity. Cells of the myocardium with no muscle origin can participate in this type of cell junction.[24,27]

The zone of the lateral sarcolemma comprises the part of the sarcolemma which is not included in the intercalated disc. This part of the membrane is the location of specialized zones with key significance for the homeostasis of the cardiomyocyte. These zones are called costameres. These are substructures of the sarcolemma, conjunct to the contractile elements of the cardiomyocyte and forming a pericellular net.[28] The costameres are sites for connection of several cytoskeletal nets, forming a “communication centre” between the extracellular matrix, the sarcolemma and the Z-disc.[28,29] The costameres are built of dystrophin and dystrophin-associated glycoprotein complex, focal adhesion complex and spectrin complex.[7,30] Another important component of the lateral sarcolemma, located on the level of the Z-disc, is the transverse tubular system or T-system.[31] The T-system consists of folds of the sarcolemma, entering deep in the inside of the cell, which allow a fast and even transmission of the action potential of the membrane towards the muscle fibre. Potassium channels and their subunits, minK, as well as some of the described proteins of the lateral sarcolemma are located in the T-system.[32]

The cytoskeleton is a structure that ensures mechanical support, thus providing arrangement in space of the other subcellular components. It maintains the structural and functional integrity of the cardiomyocyte. In addition, some of the cytoskeleton's components take part in other cell processes such as hypertrophy, cell division, migration, intracellular vesicular transport, arrangement and function of cellular components, disposition of membrane receptors and intercellular communication.[8-10,21-23] It is suggested that the cytoskeleton plays a key role in the mechanical signal transduction of the cell.[33] For an easier examination and understanding of its function, the cytoskeleton is divided, based on its morphology and topography, into different types: sarcomeric, extrasarcomeric, membrane-submembrane, nuclear.[7] Furthermore, based upon its functional characteristics, the cytoskeleton is divided into contracting part, consisting of the thick and thin filaments of the sarcomere, and non-contracting part, which takes part in dispersing the mechanical strength signal transduction and maintains the structural integrity of the cell.[27]

The sarcomeric cytoskeleton is comprised of thin and thick filaments, and Z discs. Thin filaments consist of cardiac actin, α-tropomyosin, C- , I- and T-troponins.[7,34] Actin is made up of two chains twisted one around the other, thus making up a double helix structure. Each of these chains contains spherical G-actin monomers. This way, a filiform polymer is composed – the sarcomeric F-actin.[35] One end of the thin filament is attached to the Z-disc, and the other reaches the A-zone. In the A-zone, molecular ends remain joined with the help of tropomodulin, a protein that plays a major part in keeping the correct length of thin filaments.[36] Regulatory proteins, including tropomyosin and the troponin complex are connected with actin. Thick filaments consist mainly of myosin and myosin-connecting proteins (C- , H-, and X-).[36]

Other proteins such as titin, myomesins and nebulet are also found in cardiomyocytes.[7] Titin has the biggest

protein molecule. It is connected with myosin through myosin-connecting proteins (C- and H-) as well as 1- and 2-myomesin.[34,37] Nebulet is a protein similar to nebulin in skeletal muscles. Nebulet from the cardiac muscle is shorter and does not cover up actin in its whole length.[34,37] According to some sources in literature, it takes part both in the structure and function of the Z-disc. Moreover, nebulet is important for the coupling of sarcomeric actin with α-actin and probably has importance in signal transduction pathways.[37]

Extrasarcomeric cytoskeleton comprises the intermediate desmin filaments, actin microfilaments and microtubules.[7] It executes the communication between the sarcomere on the one hand and the sarcolemma and the extracellular matrix on the other hand, through the Z-disc and the submembrane cytoskeleton.[38] Intermediate filaments are made up of desmin. They form a three dimensional skeleton that covers up the cytoplasm throughout its whole area. Those filaments have significant importance in preserving the myofibrils.[39] They coat the Z-discs and are connected to other cell organelles such as the sarcoplasmic reticulum and the T-system. Desmin filaments span from Z-discs to costameres, where they anchor through plectin and dysferlin.[40] Through plectin, desmin filaments are connected with actin filaments and microtubules. Microtubules are tubular structures that are formed as a result of the polymerization of α- and β-tubulin heterodimers. They are extremely dynamic structures of the cardiomyocyte, because of their ability to depolarize and repolarize very fast. They emit chemical and mechanical stimulus in the intra- and intercellular space.[27] Furthermore, desmin filaments promote cell stability, connecting with mitochondria, myofibrils and the Golgi apparatus.

The membrane-submembrane cytoskeleton consists of dystrophin glycoprotein complex, focal adhesion complex and spectrin complex (costamere).[7,33] The nuclear cytoskeleton (nuclear lamina) is a tight network of filaments, which functions as a supporting element of the nuclear periphery by its interaction with the nuclear membrane and the underlying chromatin.[41]

Table 1: Comparison of the morphological characteristics of atrial and ventricular cardiomyocytes and Purkinje cells

Criteria	Atrial cardiomyocytes	Ventricular cardiomyocytes	Purkinje cells
Size and shape	Elliptical cells length: 20 μm width: 6-8 μm	Long and narrow cells, often branched length: 100 μm width: 15-20 μm	Wide cells (80 μm in diameter)
Cell constitution:	Identically organized in long parallel rows		
• Myofibrils • Mitochondria	Lesser quantity comapred to ventricular cardiomyocytes	Greatest quantity	Lesser quantity comapred to ventricular cardiomyocytes
• Glycogen	Found between myofibrils and mitochondria		Ample, located between myofibrils and under sarcolemma
• Granules	„Atrial granules": composition and function is unknown	„Residual bodies" with high catecholamine content	
• T-tubular system	None	Abundant	None
• Sarcoplasmic reticulum	Fully developed and abundant in all cell types		
Intercellular junctions:			
• Intercalated discs	Short, horizontally orientated along the cell's long axis; short, perpendicularly orientated discs can be rarely seen	Specific foot-like configuration with non-specialised zones, horizontally orientated along the long axis of the muscle fibre and specialised zones, perpendicularly orientated along to the long axis of the muscle fibre; cells connect end-to-end	Oblique and zigzag direction; greatest quantity of specialized junctions with biggest overall area compared to other cell types
• Lateral junctions	Main localization of intercellular junctions	Rarely seen	Abundant

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