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Embryonic development of the proepicardium and coronary vessels

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ABSTRACT In the last few years, an increasing interest in progenitor cells has been noted. These cells are a source of undifferentiated elements from which cellular components of tissues and organs develop. Such progenitor tissue delivering stem cells for cardiac development is the proepicardium. The proepicardium is a transient organ which occurs near the venous pole of the embryonic heart and protrudes to the pericardial cavity. The proepicardium is a source of the epicardial epithelium delivering cellular components of vascular wall and interstitial tissue fibroblasts. It contributes partially to a fibrous tissue skeleton of the heart. Epicardial derived cells play also an inductive role in differentiation of cardiac myocytes into conductive tissue of the heart. Coronary vessel formation proceeds by vasculogenesis and angiogenesis. The first tubules are formed from blood islands which subsequently coalesce forming the primitive vascular plexus. Coronary arteries are formed by directional growth of vascular protrusions towards the aorta and establishing contact with the aortic wall. The coronary vascular wall matures by attaching smooth muscle cell precursors and fibroblast precursors to the endothelial cell wall. The cells of tunica media differentiate subsequently into vascular smooth muscle by acquiring specific contractile and cytoskeletal markers of smooth muscle cells in a proximal - distal direction. The coronary artery wall matures first before cardiac veins. Maturity of the vessel wall is demonstrated by the specific shape of the internal surface of the vascular wall.

KEY WORDS: *epicardium, hematopoiesis, vasculogenesis, coronary vessel development, mesenchymal cell*

Introduction

The tissues and organs of multicellular organisms develop from the pool of undifferentiated progenitor cells. The example of such a pool of progenitor cells is vertebrate proepicardium, which contains cells for the development of the heart. The proepicardium is a transient organ which is located near the venous pole of the embryonic heart and protrudes to the pericardial cavity (Virágh *et al.,* 1993). The current data on coronary vessel development indicate that the proepicardium is a source of the epicardial epithelium (Männer *et al.,* 2001), which participates in the formation of the vascular wall containing endothelial cells, smooth muscle cells and fibroblasts (Männer *et al.,* 2001). The proepicardium is also a source of cells forming connective tissue of the heart valves and modulating formation of the heart skeleton (Winter, Gittenberger-de Groot 2007). The proepicardium is described as the "organ" because it is a source of a heterogenous population of progenitor cells, which participate in the development of various structures of the heart. These cells participate in such processes as vasculogenesis, angiogenesis, morphogenesis and remodeling of extracellular matrix.

Formation of the proepicardium

The primordium of the proepicardium is found on the surface of the septum transversum in mammals, and on the surface of the sinus venosus near the embryonic liver in birds. The proepicardium primordium forms as the multicellular protrusion of the pericardial serosa covering both horns of the sinus venosus (in mammals) or the right sinus horn (in birds) (Männer *et al.,* 2001, Schulte *et al.,* 2007). These protrusions contain hyaluronic acid and a small amount of fibronectin (Kálmán *et al.,* 1995; Männer *et al.,* 2001). At this stage of development, in mammals, the embryonic liver grows into the septum transversum. In birds, which lack the

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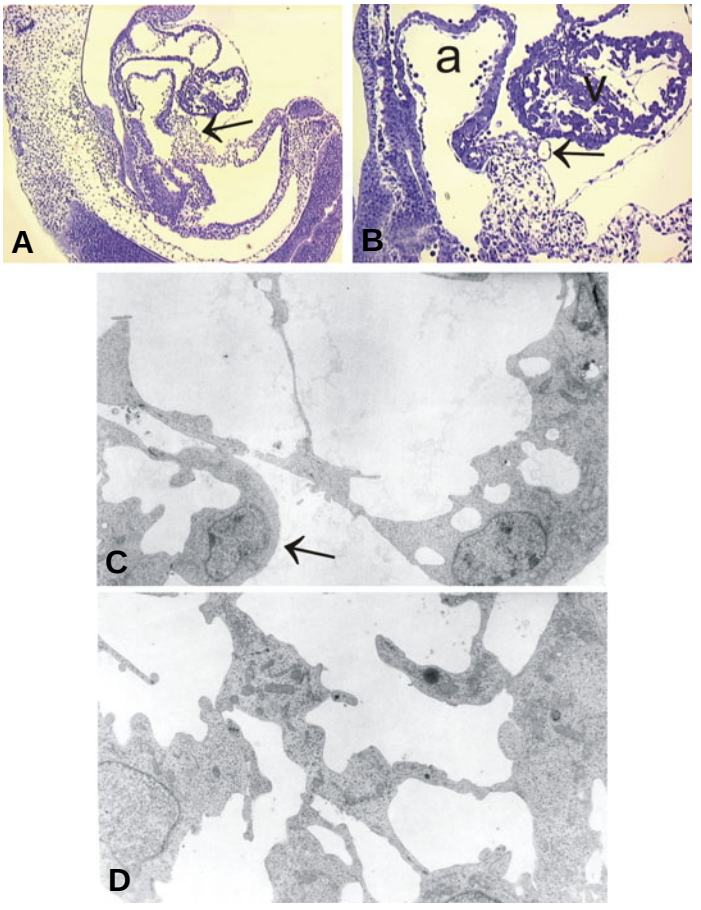

Fig. 1. The proepicardium of a mouse embryo at stage 9.5 dpc. (A) *A sagittal section of the embryo showing the location and the structure of the proepicardium (arrow), mag. 50x.* **(B)** *The proepicardium releasing a vesicle (arrow), mag. 90x.* **(C)** *Transmission electron microscopy image of the proepicardial surface with epithelial-like cells (arrow), mag. 2600x.* **(D)** *Transmission electron microscopy image of the proepicardium with loosely arranged mesenchymal cells; mag. 3300x; a, atrium; v, ventricle.*

diaphragm and in which the septum transversum does not develop, the proepicardium forms near the embryonic liver. Thus, the protrusion forms in the pericardial cavity, near the septum transversum (in mammals) or fetal liver (in birds). It resembles cauliflower (in birds) and grapes (in mammals) (Ho and Shimada 1978; Virágh, Challice 1981). These protrusions give rise to the proepicardium. In the next step of proepicardium development these protrusions grow within the pericardial cavity (Fig. 1A). In birds such as quail or chicken the proepicardium reaches about 200μm in diameter, in mice - about 60-80 μm and is correspondingly larger in human. It reaches its maximum diameter at the 4-5 week of the prenatal life in human, at 9.5 dpc in mice and at HH15-17 in birds (Hamburger and Hamilton 1951).

Polish scientist Kurkiewicz was the first who described the proepicardial protrusions (he called them villi), and suggested that they give rise to the epicardium (Kurkiewicz 1909). The proepicardium is covered with embryonic epithelial cells while the core of proepicardium constists of undifferentiated mesenchymal cells, loosely arranged and connected by intercellular junctions (Fig. 1 B,C). Further development of the proepicardium after it reaches its maximum size differs in mammals and in birds but in both cases, proepicardium differentiates into the epicardium (Virágh, Challice 1981; Hiruma, Hirakow 1989).

Immunohistochemical and genetic markers of the proepicardium

Early proepicardium does not express any markers of differentiated endothelial cells or their precursors such as angioblast (CD34, PECAM-1, VEGFR1, VEGFR2), fibroblast, hematopoietic cells (CD45, CD34), smooth muscle cells (alpha-actin) or other markers (Fig. 2). Endothelial cells are detected in the proepicardium in later stages of development when the proepicardium reaches the heart surface and attaches to it (Kattan *et al.,* 2004; Guadix *et al.,* 2006).

On the other hand the proepicardium contains the transcription factor of Wilm's tumor (WT-1) supressor gene, retinaldehyde dehydrogenase2 (RALDH2) (Pérez-Pomares *et al.,* 2002b), and *Tbx* (Hatcher *et al.,* 2004) and *Cfc* gene products (Schlueter *et al.,* 2006). Mutant mice for *WT-1* display abnormalities in the epicardium formation with a reduced number of subepicardial mesenchymal cells (Moore *et al.,* 1999). Normal development of the proepicardium requires expression of BMP factor (Schlueter *et al.,* 2006) and GATA4 transcription factor (Watt *et al.,* 2004).

Formation of the epicardium

In embryonic stages of HH14 and HH17 in birds (and at 9-10 dpc in mice) through further bending of the head region of the embryo and concomitant heart looping the dorsal surface of the heart loop approaches the ventral wall of the sinus venosus. In birds, the growing protrusions of the proepicardium approach the dorsal surface of the heart and attach to it. This forms a bridge in the pericardial cavity, between the ventral surface of the sinus venosus and dorsal wall of the heart (Männer 1993). In birds this bridge is composed of extracellular matrix (ECM) including heparan sulfate and fibronectin (Ho, Shimada 1978; Männer 1992; Männer 1993; Virágh *et al.,* 1993; Männer 1999; Nahirney *et al.,* 2003). The bridge was also observed in mammals (Nesbitt *et al.,* 2006) and its composition is similar. Looping heart is covered by cells deriving from the proepicardium, which form a simple squamous epithelium i.e. the epicardium. Initially these cells adhere to the dorsal surface of the heart. Then cells of the epicardium proliferate and spread on the heart surface (Hiruma, Hirakow 1989).

In mammals, formation of the epicardium from the proepicardium, occurs via a release of free-floating vesicles into the pericardial cavity. These vesicles are released from the tip of the proepicardium (Virágh, Challice 1981; Kuhn, Liebherr 1988; Van den Eijnde *et al.,* 1995). In mice, the highest number of free-floating vesicles in the pericardial cavity occurs at 9.5-10 dpc. These vesicles reach also the dorsal surface of the heart, attach to it, and subsequently flatten and spread on the surface of the "naked" heart. Expansion of the epicardium occurs by proliferation of proepicardial and mesothelial cells. (Fig. 3).These cells express cytokeratins, which are the marker of epithelial cells (Vrancken Peeters *et al.,* 1995). There is a distinct spatial-temporal pattern of the spreading of the mesothelial cells on the surface of the heart. The apical surfaces of the proepicardial clusters bleb off from the proepicardium, attach to the dorsal wall

of the atrioventricular sulcus and become the epicardial mesothelium. Subsequently the epicardium covers almost the whole dorsal surface of the ventricle moving towards the atrium and bulbus cordis, with a delay in the covering of the ventral surface. Beginning from the dorsal surface of the heart, the epithelial cells form the rings in the atrioventricular and ventriculo-bulbar sulcuses, subsequently cells spread to the right and to the left around the looping heart on the ventricular surface (the heart is U-shaped at this stage of development). Then, cells spread cranially and caudally reaching the outflow tract and atria (the shape of the heart resembles an "S")(Hiruma, Hirakow 1989; Vrancken Peeters *et al.,* 1995). The front line of the spreading epicardial cells covering the looping heart has a semilunar shape. The cells expand on the heart surface radially from the atrioventricular sulcus. In mice, by the end of 11 dpc, the heart is entirely covered by the epicardium. This corresponds to the stage HH26 in birds. In chicken, covering the heart with the epicardium takes 2 days.

All proepicardial cells transform into the cells of epicardium. The proepicardium disappears by the end of the 5th week of human development (in mice by 10.5-11 dpc). The limited release of cells is observed in a group of mesothelial cells from the dorsal surface of the pericardial cavity in several animal species (Männer *et al.,* 2001). We noticed such cell release in the pericardium of mouse embryos (Fig. 4).

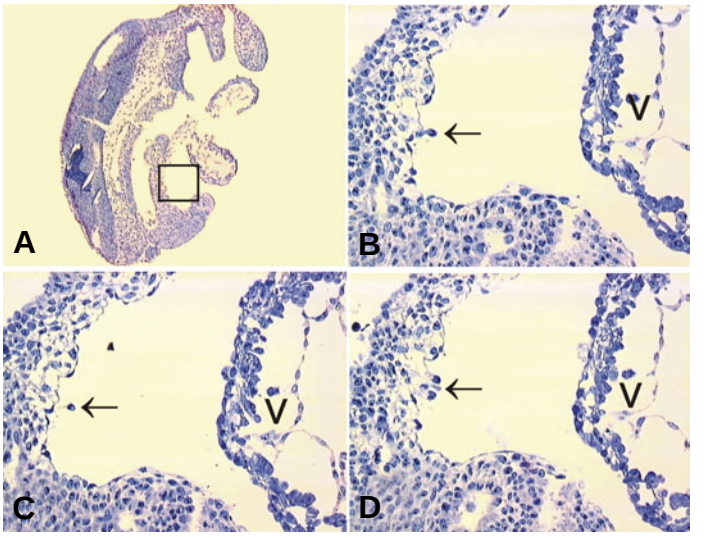

Fig. 4. Mouse embryo at the stage of 9.5 dpc. *The dorsal surface of the peritoneal-pericardial cavity with released epithelial cells, which form vesicle-like structures similar to those observed in the proepicardium.* **(A)** *The area in the rectangle is enlarged in (B,C,D).* **(B,C,D)** *Serial sections; v, ventricle; magnifications: (A) 60x, (B-D) 220x.*

The epithelial-mesenchymal transformation of epicardial cells

In further stages of development the epicardium forms cellular components of the subepicardium. Subepicardium synthesizes extra cellular matrix built from glycosaminoglycans, collagen, elastin, and fibronectin. This occurs through the invagination of the subpopulation of epicardial cells under the surface of the epicardium. The invaginating cells lose their epithelial character and transform into undifferentiated mesenchymal cells (Dettman *et al.,* 1998; Pérez-Pomares *et al.,* 1998). This phenomenon is called the epithelial-mesenchymal transformation (EMT). It is accompanied by expression of the Slug and Snail transcription factors, whose presence are necessary for this process (Carmona *et al.,* 2000; Cano *et al.,* 2000). Other growth factors like TGF-β1 and –2 also stimulate this process through their constitutive receptor– ALK2 (Olivey *et al.,* 2006). Mesenchymal cells are a source of extracellular matrix. Erytrocytes and angioblasts are found in newly formed subepicardium (Virágh *et al.,* 1993; Ratajska *et al.,* 2006, Tomanek *et al.,* 2006a). Erythrocytes are surrounded by angioblasts forming the primitive vascular vesicles (Fig. 5).

Similar epithelial-mesenchymal transformation occurs within the endocardial cushion tissue of the heart; here, the endocardial endothelial cells lose their epithelial character and transform into the mesenchymal cells of the cushion tissue (Markwald *et al.,* 1977).

The mesenchymal cells which derive from the epicardium (epicardial-derived mesenchymal cells - EPDC) build a layer of connective tissue, which is the thickest in the atrioventricular and interventricular sulcuses. This connective tissue will form the epicardium, which will be covered with epithelial cells and filled with fibroblasts and extracellular matrix built from collagen, glycosaminoglycans, elastic and collagen fibers. Subepicardial space is occupied by primitive vascular vesicles, which are not yet connected to the systemic circulation (Kálmán *et al.,* 1995).

The routs of migration of mesenchymal cells derived from the epicardium (and earlier from the proepicardium) are known from the analysis of interspecies chimeras, in which the quail

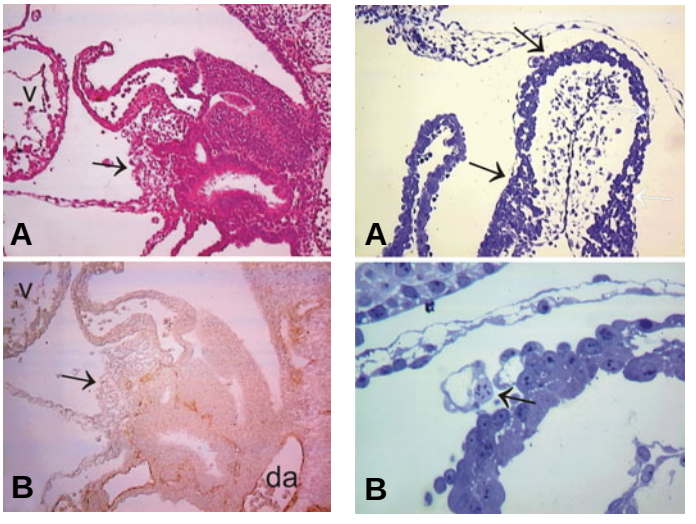

Fig. 2 (Left). The sagittal section of the proepicardium of mouse embryo at the stage of 9.5 dpc. (A) *A histological section of the proepicardium (arrow) stained with hematoxylin-eosin; numerous vesicles released to the pericardial cavity are visible.* **(B)** *Section stained with anti-CD34 antibodies; CD34 positive cells are located in the dorsal aorta and at the base of the proepicardium; v, ventricle; da, dorsal aorta. Magnification: 200x.*

Fig. 3 (Right). The heart of mouse stage of 10 dpc embryo partially covered with the epicardium. (A) *The area covered with the epicardium is located between arrows; mag. 200x.* **(B)** *Proliferating epicardial cell (arrow), mag. 380x.*

proepicardium was inserted into the chicken pericardial cavity (Gittenberger-de Groot *et al.,* 1998). The presence of quail specific nuclear marker allowed tracking the migration of quail cells in the chimeric chicken embryo. In addition, the retroviral tagging of the proepicardium with a reporter gene for β-galactosidase allowed tracking the migration of proepicardium-derived cells (Mikawa, Fishman 1992; Mikawa, Gourdie 1996).

It is not known whether there are separate populations of mesothelial cells able to differentiate into different cell types: endothelial, smooth muscle or fibroblast, or whether all EPDC are able to differentiate into all types of cells, depending on the action of specific growth factors (Dettman *et al.,* 1998; Pérez-Pomares *et al.,* 2002a). *In vitro* studies showed that the growth factors are able to induce all these transformations in certain populations of mesenchymal cells. Also the epicardial epithelium has a great capacity to differentiate into other cell types (Wessels, Pérez-Pomares 2004). The process begins with initial dedifferentiation of flat epithelial cells into mesenchymal cells. Although the proepicardium does not have differentiated cells it has an enormous potential to differentiate into vascular cells *in vivo* and *in vitro* (Guadix *et al.,* 2006, Pérez-Pomares *et al.,* 2006).

Knockout mice devoid of VCAM-1 adhesive molecule do not develop the epicardium and their myocardium is very thin. A large amount of blood cells is present within the pericardial cavity of these knockout mice (Kwee *et al.,* 1995). Photoablation experiments on chicken hearts showed that the hearts are devoid of the proepicardium and epicardium, are thin-walled and aneurysmal, have defects of coronary vasculature and develop hemo- and hydropericardium (Männer *et al.,* 2005). This indicates that the proepicardium plays a role in maintaining the mechanical properties of the heart wall.

Formation of coronary vessels

The coronary vessels develop from the blood islands which are the aggregates of endothelial cells and erythrocytes and are not connected to the systemic circulation (Hirakow 1983; Rongish *et al.,* 1994; Ratajska, Fiejka 1999). The blood islands coalesce to form the primitive vessels. In birds, at the HH23 stage, the blood islands, erythrocytes and capillary vessels are most numerous within the subepicardial space (Hiruma, Hirakow 1989) and the vascularization of the heart is proceded by the development of the epicardial cover. In birds and mammals, the earliest vascular structures are visible within the sinus venosus and the dorsal part of the atrioventricular sulcus – which is the area of developing heart first being covered by the epicardium (Vrancken Peeters *et al.,* 1997b; Kattan *et al.,* 2004).

The origin of the erythrocytes within the blood islands is another interesting issue. It has been suggested that the erthrocytes and angioblasts derive from a common precursor cells – hemangioblasts (Pardanaud *et al.,* 1989; Muñoz-Chápuli *et al.,* 1999). Retroviral tagging of the proepicardium showed that the erythrocytes of the subepicardium derive from the proepicardial progenitor cells which differentiate into erythroblasts wthin the heart (Tomanek *et al.,* 2006a). This appears to be consistent with the results of earlier studies showing the presence of erytroblasts within the subepicardium (Virágh 1990; Kálmán *et al.,* 1995) and the recent study by Kattan (2004) demonstrating presence of progenitor cell markers (CD45) on erythroblast clusters within subepicardial area. Another possible source of erythrocytes within the blood islands, would be the systemic circulation from where they migrate to the heart and subsequently assemble with angioblasts (Ratajska *et al.,* 2006).

Retroviral tagging of the proepicardium identified the sources of smooth muscle cells of the tunica media, fibroblasts of the adventitia of large vessels, and the interstitial heart fibroblasts (Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996). All these cell types derive from the epicardial mesothelium by the epithelial-mesenchymal transformation (EMT) and subsequent differentiation (Dettman *et al.,* 1998; Gittenberger-de Groot *et al.,* 1998; Vrancken Peeters *et al.,* 1999; Pérez-Pomares *et al.,* 2002a; Muñoz-Chápuli *et al.,* 2002). Mesenchymal cells are able to infiltrate myocardium reaching the subendocardial cushions (Gittenberger-de Groot *et al.,* 1998). Depending on local availability of the growth factors such as PDGF-BB, VEGF or bFGF, the mesenchymal cells differentiate into smooth muscle cells or endothelial cells, respectively (Cox, Poole 2000; Pérez-Pomares *et al.,* 2002a).

The studies of chicken-quail chimeras broadened our understanding of the precursors and development of the coronary vessel. There are two main theories on the origin of the vascular endothelial cells. First theory, based on experiments with quail-chicken chimeras in which small pieces of quail liver were transplanted into the chicken coelom, claims that vascular endothelial cells derive from liver primordium, and the angioblasts differentiate within the subepicardium which subsequently gives rise to the primitive subepicardial vascular plexus (Poelmann *et al.,* 2002). The second theory maintains that epicardium-derived mesenchymal cells (EPDC) are able to differentiate into vascular endothelial cells (Männer 1999; Muñoz-Chápuli *et al.,* 2002; Pérez-Pomares *et al.,* 2002a). The latter theory is supported by the results of the retroviral labeling of the proepicardium, and by the chicken-quail

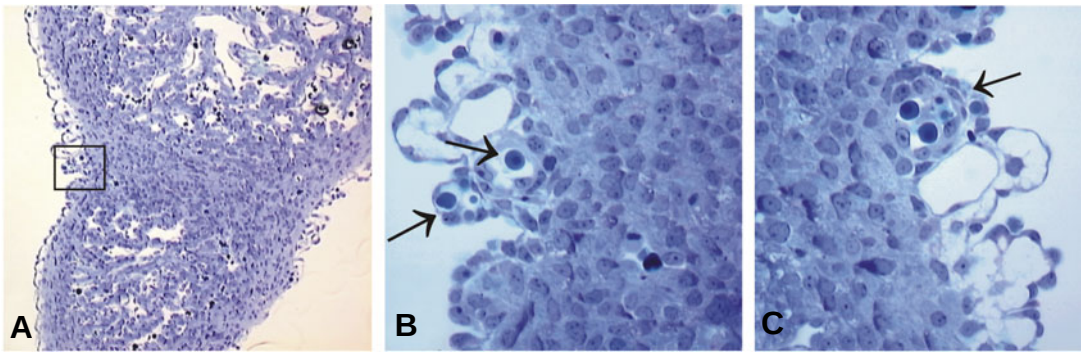

Fig. 5. The epicardium of a mouse heart at stage 13 dpc. *Vascular vesicles located in the subepicardial space contain nucleated red blood cells (arrows).* **(A)** *Section of the ventricles and the interventricular sulcus; the area in the rectangle is enlarged in* **(B)**, *mag. 150x.* **(C)** *Vascular vesicle in the interventricular sulcus. These vesicles are precursors of subepicardial coronary vessels. Mag. 380x in (B,C) .*

chimera studies. Double labeling of EPDC with anti-QH1 antibodies and cytokeratin showed that both markers were present in the precursors of the coronary endothelial cells. Additional support for this theory comes from the interspecies comparison of epicardially derived cells. In dogfish (*Scyliorhinus canicula*) all subepicardially derived cells originate from the epicardial mesothelium. In this species subepicardial vessels arise long before the migration of extracardiac angioblasts begins (Muñoz-Chápuli *et al.,* 1996). The experiments in rats in which avascular hearts were grafted *in oculo* showed that the coronary vessels in transplanted hearts develop without any vascular contribution from the host (Rongish *et al.,* 1994). Both studies are in agreement with the idea that splanchnic angioblast differentiation occurs *in situ* without significant migration of vascular cell precursors (Drake *et al.,* 1997). The idea that the angioblasts originate *in situ* from the splanchnic mesoderm is also supported by the studies of 5-somite stage avian embryo (Cox and Poole 2000; Poole *et al.,* 2001).

As we mentioned previously, the blood islands are precursors of coronary vasculature. Blood islands differentiate into vascular tubes (capillaries), which appear first within the subepicardium. Capillaries grow within the subepicardium of the atrioventricular and interventricular sulcuses and coalesce forming primitive vascular network. Formation of the coronary arteries commences with directional growth of the protruding capillaries towards the sinuses of Valsalva and establishing patency with the aortic lumen (Bogers *et al.,* 1990; Waldo *et al.,* 1990). The protrusion of primordia of coronary arteries towards the aorta is facilitated by apoptosis of the aortic wall (Bernanke, Valkey 2002; Tomanek *et al.,* 2006b). Connection with the aorta is established in rats on ED16 (Heinzberger 1983), at HH stage 29-35 in chicken and quail hearts (Kattan *et al.,* 2004), and at 44-49 day in human heart (Conte, Pellegrini 1984; Hutchins *et al.,* 1988; Mandarin-Lacerda 1990). Interestingly, there are several capillaries penetrating towards the three sinuses of Valsalva but only two (in the left and right sinuses, respectively) establish connection with the aorta (Poelmann *et al.,* 1993). Capillary plexus spreads around the outflow tract and within the myocardium of diaphragmatic surface of the heart. Subsequently, the plexus grows on the sternal surface of the heart. The left ventricle undergoes vascularization followed by the vascularization of the right ventricle. The apex of the heart vascularizes as the last (Rychter *et al.,* 1975, Vrancken-Peeters *et al.,* 1997b, Kattan *et al.,* 2004). Initially there is a gradient of the myocardial wall vascularization with the highest density of vessels in the subepicardium and the lowest in the subendocardium. This is consistent with the expression of VEGF and its receptor-2 (Tomanek *et al.,* 1999a; 2002; Tomanek, Zheng 2002). It is believed that the thickening of the myocardial wall (Tomanek *et al.,* 1999b), and relative hypoxia in the subepicardium are the stimuli responsible for the rapid development of coronary vessels in the subepicardium. Multiple growth factors participate in the development and spreading of the vasculature (Ratajska *et al.,* 1995; Tomanek *et al.,* 1998; 2001). We do not known what signal allows the directional growth of coronary artery precursors towards the aorta. Probably the aorta is recognized by at least one signal from growth factors or from parasympathetic ganglia situated at the roots of the aorta (Hood, Rosenquist 1992). The parasympathetic ganglia which are derivative of neural crest cells (Verberne *et al.,* 1998) are positioned closely to coronary artery ostia in chick heart (Waldo *et al.,* 1994). This suggests that the

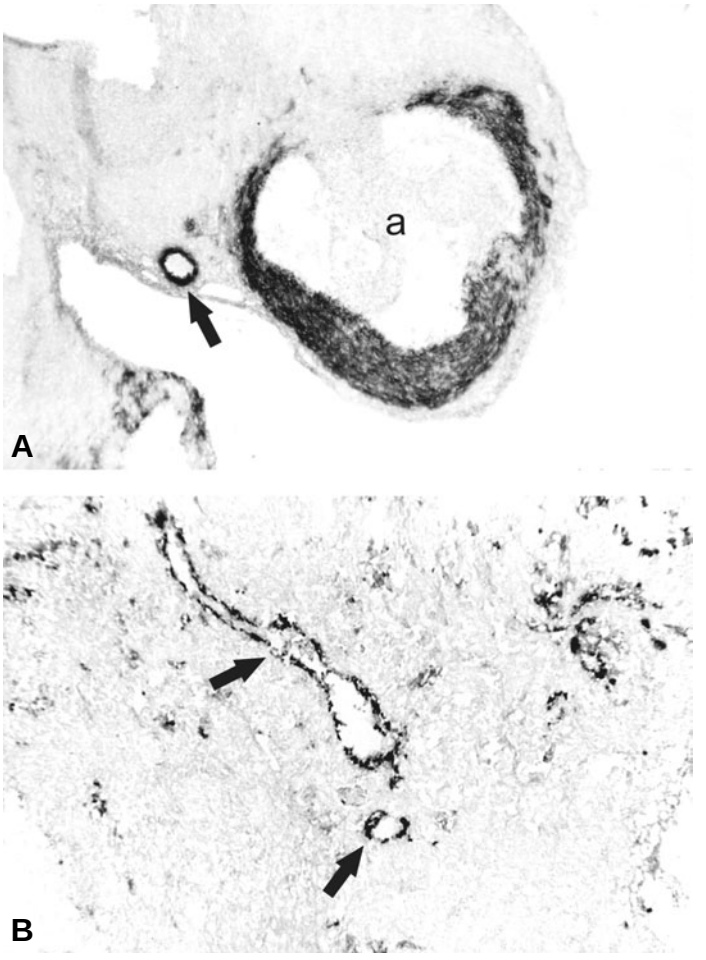

Fig. 6. Rat heart of 18 dpc embryo. *Immunostaining with anti-smooth muscle myosin antibody (A,B) smooth muscle myosin is present within the proximal* **(A)** *and distal wall* **(B)** *of coronary artery.*

signal(s) from the parasympathetic ganglia may contribute to the formation of coronary ostia. In cases such as Bland-White-Garland syndrome, this signal is imprecise allowing the primordia to grow towards the pulmonary trunk (1933). In congenital heart malformations, however, with dextroposition of the aorta relative to the pulmonary trunk, the primordial coronary arteries grow towards the aorta (Gonzáles-Iriarte *et al.,* 2003; Ratajska *et al.,* 2005).

Formation of the tunica media of larger coronary vessels starts at the proximal end of coronary artery by building up a layer of mesenchymal cells. These cells approach the vascular wall before the connection of coronary artery precursors with the aorta is established (Ratajska, Fiejka 1999). Further differentiation into smooth muscle cells commences when blood starts to flow within the patent coronary system (Hood, Rosenquist 1992). The differentiation into smooth muscle cells requires acquisition of certain contractile and cytoskeletal proteins, which occurs sequentially and is species-specific (Owens *et al.,* 1995; Hungerford, Little 1999; Ratajska *et al.,* 2001). The first is the smooth muscle alpha actin, which appears sequentially from the proximal to distal part of the coronary vessel wall (Hood, Rosenquist 1992, Ratajska *et al.,* 2001). Subsequently, the smooth muscle myosin appears

(Fig. 6). Concomitantly with the start of blood flow within the patent coronary system, the shear stress on endothelial cells increases stimulating a release of certain substances from the epithelial cells (Hannan *et al.,* 1988; Joseph-Silverstein *et al.,* 1987). These substances modulate smooth muscle cell differentiation and influence the smooth muscle cell proliferation (Sterpetti *et al.,* 1993). An increase in shear stress within the arterial side of the coronary system and rapid blood flow in arterial wall explains why the vascular wall of arterial system differentiates earlier than the cardiac veins (Vrancken Peeters *et al.,* 1997a). In addition, the specific shape of the internal wall of coronary vasculature of the embryonic heart can be explained by the elevated speed of blood flow within the arteries as compared to veins (Ratajska *et al.,* 2003). The development of the coronary vessel is not restricted to the embryonic period of life and it continues postnatally. It is accomplished by remodeling of the vasculature: spreading of existing vessels by sprouting, intussusceptive growth and disappearing of the branches connecting the vessels (Tomanek 2005).

The role of the epicardium in myocardial development

Formation of the epicardium is necessary for normal development of myocardium, its "tightness", stiffness, elasticity, and for the proper vascularization of the heart wall. The heart devoid of the proepicardium (as the result of photoablation) develops malformations such as double outlet right ventricle, and deficiency of the interventricular septum in its membranous part (Männer *et al.,* 2005). It is highly probable that EPDC takes part in the formation of the interventricular septum. It is not known however, in what way EPDC could induce the complex pathological changes of the heart such as double outlet right ventricle (Männer *et al.,* 2005). Mice devoid of *RXRalpha* gene exhibit delayed development of the epicardium. Their epicardium exhibits malformations with exuberant extracellular matrix and increased apoptosis of mesenchymal cells (Jenkins *et al.,* 2005). These mice have lower number of subepicardial mesenchymal cells. In addition, the fibronectin aggregation in their subepicardium is abnormal (Jenkins *et al.,* 2005

The mesenchymal cells (deriving from the epicardial cells) are able to migratefrom the epicardium to the subendocardial area where they differentiate into fibroblasts, which synthesize extracellular matrix. These mesenchymal cells migrate to the subendocardial area where they form heart valves. They also play an active role in the formation of the fibrous heart skeleton by inducing cardiomyocyte-fibroblast transformation, which occurs in areas of insulation of atrial and ventricular myocardium (Winter, Gittenberger-de Groot 2007).

The recent studies on chicken embryos, indicate an inductive influence of EPDC on those cardiac myocytes that differentiate into the conductive system of the heart (Gittenberger-de Groot *et al.,* 2003) and the periarterial and subendocardial Purkinje fibers (Gourdie *et al.,* 1998,1999). EPDC are also able to differentiate into smooth muscle cells of the tunica media and fibroblasts of the adventitia (Vrancken Peeters *et al.,* 1999). This suggests that the inductive influence of EPDC on Purkinje fiber differentiation is indirect and does not require a direct contact with the arteries and periarterial Purkinje fibers. *In vitro* studies demonstrated that endothelin cascade induces Purkinje fiber differentiation (Gourdie *et al.,* 1998). This has been confirmed by *in vivo* studies (Takebayashi-Suzuki *et al.,* 2000), which showed that the communication between EPDC and primitive cardiocytes is mediated via endothelins released from EPDC. However, in birds, the EPDC play a more direct role in differentiation of periarterial and subendocardial Purkinje fibers of the heart.

In some rare cases the epicardial mesothelium undergoes a malignant transformation into a tumor – mesothelioma. It is a very rare tumor, which derives from membranous serosa of body cavities, and rarely from pericardium or from the epicardium (Hammar 2006). In the latter case, the tumor grows into the pericardial cavity and surrounds the heart. Microscopically, this tumor consists of epithelial cells, which sometimes form canalicular, glandular or papillomatous structures.

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