Virchows Arch (1995) 427:213–221

ORIGINAL ARTICLE

Tadashi Tanimoto · Yuji Ohtsuki

The pathogenesis of so-called cardiac rhabdomyoma in swine: a histological, immunohistochemical and ultrastructural study

Received: 28 April 1995 / Accepted: 26 May 1995

Abstract To determine whether cardiac rhabdomyoma (CR) is a hamartoma of fetal cardiac myocyte, we investigated five cases of CRs that spontaneously developed in five 6-month-old hybrid swine with histological, immunohistochemical, and ultrastructural techniques. The cases were four multiple and one solitary neoplasms, which appeared as intraventricular nodules of various sizes without any congenital malformations. Histologically, the large ovoid CR cells with an occasional spider-web appearance showed a transition from normal-looking cardiac myocytes or rarely from Purkinje cells, but no mitotic figures. Besides large amounts of glycogen, the CR cells contained many PAS-negative, large cytoplasmic vacuoles filled with eosinophilic or fibrillar substance. Immunohistochemically, the CR cells showed intense positivity for desmin and variable positivities for vimentin, α-atrial naturiuretic peptide, and proliferating cell nuclear antigen. These positivities were not seen in adjacent cardiac myocytes. Cytokeratin was negative in the CR cells but was positive in fetal cardiac myocytes of early gestation. Rod-like or granular positivity for α-actinin in the CR cells was similar to that in nemaline myopathy. Ultrastructurally, the CR cells contained myofibrils that frequently showed myofibrillar degeneration and produced large intracytoplasmic vacuoles. These myofibrils often mingled with nemaline bodies and leptofibrils that continued to the Z bands. T-systems, sarcoplasmic reticulum, and intercalated discs, which are specific features of postnatal cardiac myocytes, were sometimes observed in the CR cells. Increase of glycogen and mitochondria and appearance of atrial-specific granules associated with the Golgi apparatus were other features noted. The present findings have not been reported, even in human CR. From these new observations with the recent report on the occurrence of CR in neonatal piglets, swine CR does not belong to the entity of hamartoma but may be a congenital dysplasia of the perinatal cardiac tissues with myofibrillar degeneration, affecting mainly cardiac myocytes and rarely Purkinje cells. The various immunophenotypic changes including proliferating cell nuclear antigen and the increase and appearance of cytoplasmic elements compared with mature cardiac myocytes can be interpreted as reactive or regenerative changes due to myofibrillar degeneration.

Key words Cardiac rhabdomyoma · Dysplasia · Hamartoma · Myofibrillar degeneration · Swine

Introduction

The term "cardiac rhabdomyoma (CR)" has been applied to the intracardiac nodules consisting of large, ovoid, and glycogen-filled striated muscle cells with an occasional spider-web appearance [2]. Because of this nomenclature, the lesion is usually categorized under cardiac neoplasms or rhabdomyoma, although its identity has been unclear [24, 27].

In humans, primary cardiac neoplasms are rare, but CR is the most common one detected in infancy and childhood. It develops as multiple intracavitary or intramural nodules found mostly in the ventricles, undergoes spontaneous regression, and is frequently associated with tuberous sclerosis and other congenital malformations and neoplasms [2, 11, 16, 27]. For these reasons with the histological and ultrastructural findings, most investigators have proposed that CR is a hamartoma of fetal cardiac myocyte (synonymous with developmental abnormality, congenital malformation, or arrested cardiac myocyte maturation) [17, 34, 35]. Other speculations have included a neoplasm of cardiac myocyte or Purkinje cell (Purkinjeoma), glycogen storage disease, cellular gigantism, and dysplasia [2, 5, 13].

Neoplasms of striated muscle are rare in any of the domestic animals, and about one-third of them are CRs

T. Tanimoto
Chuo Meat Inspection Center, Kochi Prefectural Government, Ebinomaru, Kochi City 780, Japan

Y. Ohtsuki (✉)
Department of Pathology II, Kochi Medical School, Kohasu, Oko, Nankoku City 783, Japan

in swine, cattle, sheep, and dogs. The incidence of swine CR is believed to be highest in domestic animals [23, 24], although a few case reports have been reported [5, 31]. Recently, a breed predisposition of CR was reported in red wattle and red wattle-cross bred swine [28].

To determine whether CR is a hamartoma of fetal cardiac myocyte, we think combined immunohistochemical and ultrastructural studies are important and effective. However, this approach has not been attempted in domestic animals as well as humans because of the low incidence of this lesion and the use of inadequate fixatives for specimens. In this paper, we described some new observations regarding the pathogenesis of CR in swine from our histological, immunohistochemical, and ultrastructural findings.

Materials and methods

Five swine CRs were incidentally obtained at a meat inspection facility in Nakamura City (Kochi, Japan) from 1983 to 1992.

For histological studies, samples (including other organs) were fixed in 20% neutral-buffered formalin for 24 h or methacarn fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid) [39] for 4 h at room temperature, and embedded in paraffin. Sections cut at 3–5 μm were stained with haematoxylin-eosin-erythrosin (HE), Masson's trichrome, periodic acid-Schiff (PAS) with or without diastase digestion, and phosphotungistic acid-haematoxylin (PTAH). Only specimens stained with PAS were prepared from methacarn-fixed materials.

Table 1 Clinical data and tumour distribution in cardiac rhabdomyomas in five swine (*M/c*, castrated male; *F*, female; *LV*, left ventricle; *VS*, ventricular septum; *RV*, right ventricle; >, an order of the frequency of the tumour nodules)

Case no.	Breed	Age (years)	Sex	Tumour distribution
1	Hybrid	0.5	M/c	LV>VS (diffuse)
2	Hybrid	0.5	F	LV>VS>RV (diffuse)
3	Hybrid	0.5	F	LV (single)
4	Hybrid	0.5	F	LV>VS>RV (diffuse)
5	Hybrid	0.5	F	LV>VS>RV (diffuse)

For immunohistochemical studies, we adopted the avidin-biotin-peroxidase complex (ABC) method using paraffin-embedded sections and the primary antibodies reactive with striated muscle cells and their neoplasms (Table 2) [14, 24, 29, 36, 39, 40]. To investigate proliferating activities of the CR cells, staining for proliferating cell nuclear antigen (PCNA) was also done. The immunoreactivity of the CR cells was compared with that of swine cardiac myocytes from five adults (6 month), five newborns (10 day), 20 fetuses (30, 60, 90, or 110 days), and five adults (6 month) with severe myocarditis. Gestation of swine is within a period of 112 to 115 days. Methacarn or formalin-fixed, paraffin-embedded tissue sections were used and the choice for each antibody is also listed in Table 2. Briefly, deparaffinized sectioned were incubated with 1% hydrogen peroxide in methanol for 30 min to inhibit endogenous peroxidase and then rinsed in phosphate-buffered saline (PBS). Non-specific staining was blocked by incubation with 10% normal rabbit or goat serum for 10 min and then the primary antibodies were applied for 24 h at 4 °C. This was followed by processing with an ABC staining kit (Histofine SAB-PO kit, Nichirei, Tokyo, Japan), employing incubation with a biotinylated second antibody (rabbit anti-mouse or goat anti-rabbit) for 10 min at room temperature and incubation with a peroxidase-conjugated streptoavidin for 5 min at room temperature. Each staining step was done after washing three times with PBS for 5 min. Colour was developed for 3 min with 0.02% 3,3′-diaminobenzidine in PBS containing 0.02% hydrogen peroxide. The sections were counterstained with Mayer's haematoxylin for 1 min, dehydrated, and mounted. Negative controls omitting the first antibodies were treated as described above.

For ultrastructural studies, samples taken from different areas of the intraventricular nodules were cut into small pieces, fixed in 2.5% phosphate-buffered glutaraldehyde solution for 2 h, and post-fixed in 1% phosphate-buffered osmium tetroxide solution for 1 h at 4 °C. Then the samples were embedded in Epon 812 in a routine manner. Ultrathin sections were cut, doubly stained with uranyl acetate and lead citrate, and examined with an electron microscope (H-300 type, Hitachi, Tokyo, Japan).

Results

The clinical data and the tumour distribution in each swine are summarized in Table 1. The pathological findings of all the cases were essentially similar, and their details are described below. All the swine had no physical abnormalities such as pulmonary congestion or cyanosis. The prevalence of the CRs was 0.55/100,000 inspected swine.

Table 2 Antibodies used for immunohistochemical studies of swine cardiac rhabdomyomas (*CPK-MM*, creatine phosphokinase-MM; *ANP*, atrial natriuretic peptide; *PCNA*, proliferating cell nuclear antigen; *GFAP*, glial fibrillary acidic protein; *M*, methacarn-fixed sections; *F*, formalin-fixed sections)

Antibody	Clone	Application	Dilution	Source/Reference No.
Muscle actin	HHF-35	M	1:24,000	Enzo Biochem Inc., New York, NY
α-sarcomeric actin	Alpha-Sr-1	M	1:100	Dako Japan Co. Ltd., Kyoto, Japan
α-smooth muscle actin	IA4	M	1:3,000	BioMakor Ltd., Rehovot, Israel
α-actinin	BM-75.2	M	1:2,000	BioMakor
Desmin	D33	M	1:100	Dako
Fast myosin	29-1D12	M	1:300	Amersham, Tokyo, Japan
Myoglobin	Polyclonal	F	1:1,000	Dako
CPK-MM	Polyclonal	F	1:2,000	Ventrex Lab. Inc., Portland, ME
Vimentin	V9	M	1:100	Dako
α-ANP	Polyclonal	F	1:10,000	29, 37
PCNA	PC10	M	1:1,000	Dako
GFAP	Polyclonal	M	1:500	Dako
Neurofilament	2F11	M	1:500	Dako
Cytokeratin	CAM 5.2	M	1:50	Becton Dickinson Nippon, Tokyo, Japan
Cytokeratin	AE1/AE3	M	1:500	Boehringer Mannheim GmbH, Mannheim, Germany

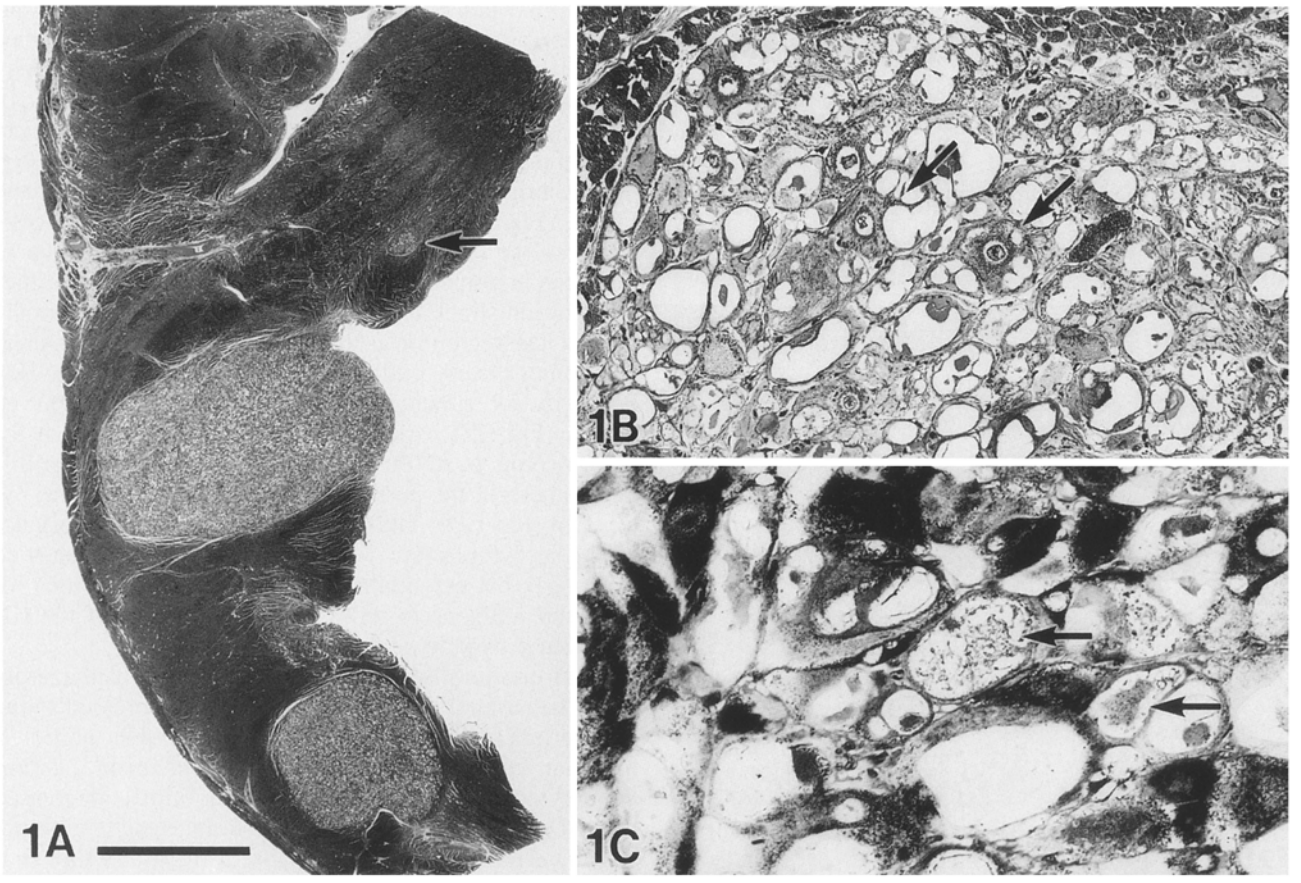

Fig. 1A–C General histology of a cardiac rhabdomyoma in swine (Case no. 1). **A**, Low power view of the subendocardial nodules protruding into the lumen of the right ventricle. An *arrow* shows a barely visible focus. HE. Bar=0.5 cm. **B**, Large, ovoid CR cells possessing various-sized cytoplasmic vacuoles. Note the CR cells showing "spider-web" appearance (*arrows*). HE. ×150. **C**, Many intracytoplasmic vacuoles containing fibrillar materials (*arrows*) with large amounts of glycogen in the CR cells. Methacarn-fixed section, PAS. ×300

Macroscopically, the CRs were yellow to brown in colour, usually well-defined multiple nodules less than 1 cm in diameter. The CR nodules were found in the subendocardium and protruded into the lumen or subepicardial and spread into the endocardium of the left ventricle, ventricular septum, and right ventricle in order of frequency (Fig. 1A). Case no. 3 had a solitary subendocardial nodule in the left ventricle that measured 3 cm in diameter. No CR nodules were found in the atrium or the cardiac valves. Other organs including the brain had no apparent abnormalities.

Histologically, the CRs were circumscribed but not encapsulated and compressed the adjacent normal cardiac myocytes. The CR tissue contained vessels as seen in the normal heart. The large ovoid CR cells contained varying amounts of myofibrils with cross striations (which were shown more clearly with PTAH) and showed varying degrees of cytoplasmic vacuolation. Their large oval nuclei were usually single but were sometimes binucleated with an irregular contour that contained a few prominent eosinophilic nucleoli (Fig. 1B). No mitotic figures were seen. The large, non-vacuolated CR cells resembled the Purkinje cells (figure not shown). Occasionally, the CR cells showed "spider-web" appearance, characterized by a centrally located cytoplasmic mass containing the nucleus and giving off slender cytoplasmic projections (Fig. 1B). Cytoplasmic vacuoles were filled with diastase-labile, PAS-positive material that proved to be glycogen (Fig. 1C). However, even with methacarn fixative, many large PAS-negative vacuoles containing eosinophilic or fibrillar substance were present (Fig. 1C). At the periphery of some part of the nodules, apparent transitions from normal-looking cardiac myocytes to the CR cells (Fig. 2A) were observed that characterized by cloudy swelling of the cytoplasm, loss of myofibrils, cytoplasmic vacuolation, and final adoption of the features of the CR cells (Fig. 2B). Isolated changes of Purkinje cells to CR cells were rarely observed (figure not shown). The necrotic CR cells with calcification were frequently observed that surrounded by lymphocytes and histiocytes with accompanying fibrosis (figure not shown).

The immunohistochemical findings for the CR cells and cardiac myocytes from adults, fetuses, and adults with myocarditis are summarized in Table 3. As for fetal cardiac myocytes of four different gestational ages, they showed essentially similar immunoreactivities. However, expressions of vimentin, PCNA, and cytokeratin

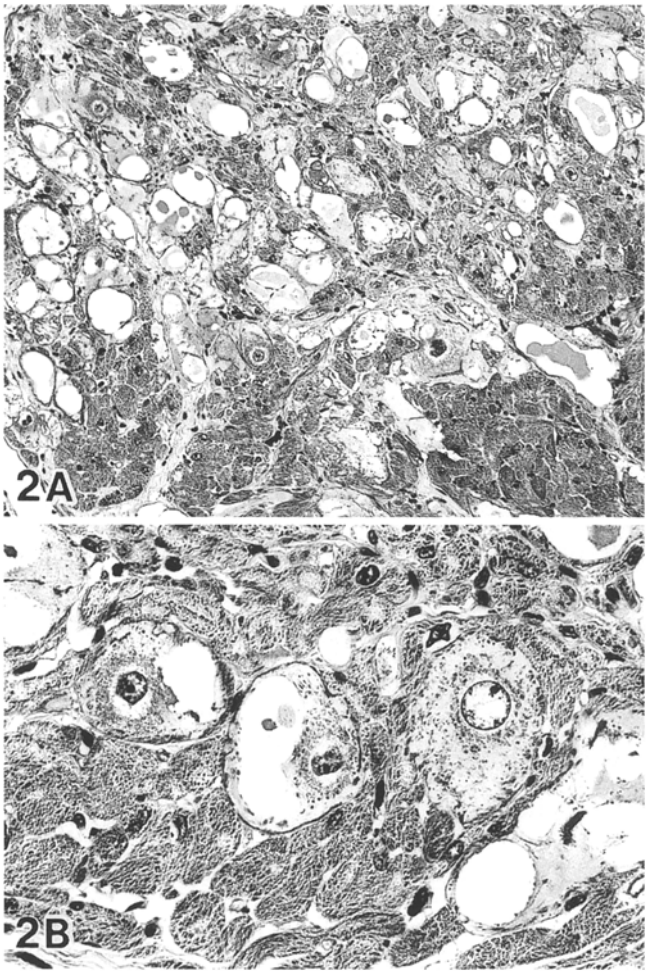

Fig. 2A, B Histology of a poorly demarcated nodule possessing transitional changes from normal-looking cardiac myocytes to the CR cells in swine (Case no. 2). **A**, An area of transitional changes. HE. ×180. **B**, Higher magnification of a transitional area. A transverse section of cardiac myocytes showing cloudy swelling, loss of myofibrils, and vacuolation of the cytoplasm. HE. ×550

AE1/AE3 were gradually diminished along with gestational age advanced (++: 30 and 60 days, ++ to +: 90 day, + to −: 110 day). The CR cells showed similar positivity and intensity as Purkinje cells, fetal cardiac myocytes, or adult cardiac myocytes with myocarditis with respect to α-actinin (Fig. 3A), desmin (Fig. 3B), vimentin (Fig. 3C), α-atrial naturiuretic peptide (α-ANP) (Fig. 3D), and PCNA (Fig. 3E). The α-actinin staining in the CR cells was rod-like or granular (Fig. 3A) which was similar to that seen in nemaline myopathy [25], but was sometimes weak or indistinct. Desmin positivity was observed in the perinuclear region or at the margins of the CR cells and sometimes clearly outlined the cross striations (Fig. 3B). Positivity for vimentin (Fig. 3C), α-ANP (Fig. 3D), and PCNA (Fig. 3E) varied markedly (++ to −) even in the same nodule. α-ANP positivity showed a granular or diffuse pattern in the perinuclear region or in the entire cytoplasm (Fig. 3D). The eosinophilic or fibrillar substances in the cytoplasmic vacuoles of the CR cells showed very weak but essentially similar immunopositivities for muscular antigens to those of the cytoplasm of the CR cells that proved to be damaged myofibrils.

Ultrastructurally, the CR cells possessed haphazardly arranged striated muscle fibres, which compressed to the cell periphery by a large amount of glycogen particles, irregular mitochondria with well-formed cristae, and/or large cytoplasmic vacuoles. As the myofibrils decreased in the cytoplasm, glycogen tended to increase (figure not shown). The fibrils were made up of thick and thin filaments that produced both A (composed of H and M) and I (composed of Z) bands, but the H and M bands were sometimes faint. The large cytoplasmic vacuoles contained electron-lucent, filamentous or granular substance, which was continuous with loose or fragmented myofibrils at the periphery of the vacuoles (Figs. 4A, B). These changes were compatible with the features of myofibrillar degeneration [20]. Hypertrophic Z bands with fragmentation, which were quite similar to the "ne-

Table 3 Immunohistochemical result of cardiac rhabdomyomas in five swine comparing with normal and diseased cardiac myocytes (*CPK-MM*, creatine phosphokinase-MM; *ANP*, atrial natriuretic peptide; *PCNA*, proliferating cell nuclear antigen; *GFAP*, glial fibrillary acidic protein; *CK*, cytokeratin; −, negative; +, positive; ++, strongly positive)

Antibodies tested	Positivity and intensity					
	Rhabdomyomas	Normal cardiac myocytes				Myocarditis
		Adult	Purkinje cells	Newborn	Fetal	
Muscle actin	+	+	+	+	+	+
α-sarcomeric actin	+	+	+	+	+	+
α-smooth muscle actin	−	−	−	−	−	−
α-actinin	++ to +	+	++	+	+	+
Desmin	++	+	++	+	+	++
Fast Myosin	+	+	+	+	+	+
Myoglobin	+	+	+	+	+	+
CPK-MM	+	+	+	+	+	+
Vimentin	++ to −	−	++	−	++ to −[c]	++ to −
α-ANP	++ to −	++[b]	++	++[b]	++[b]	++ to −
PCNA	++ to −	−[a]	−	+ to −	++ to −[c]	++ to −
GFAP	−	−	−	−	−	−
Neurofilament	−	−	−	−	−	−
CK CAM 5.2	−	−	−	−	−	−
CK AE1/AE3	−	−	−	−[a]	++ to −[c]	−

[a] Positive cells were rare
[b] Positivity was restricted to the atrial myocytes
[c] Positivity gradually diminished as gestational age advanced (++: 30 and 60 days, ++ to +: 90 day; + to −: 110 day)

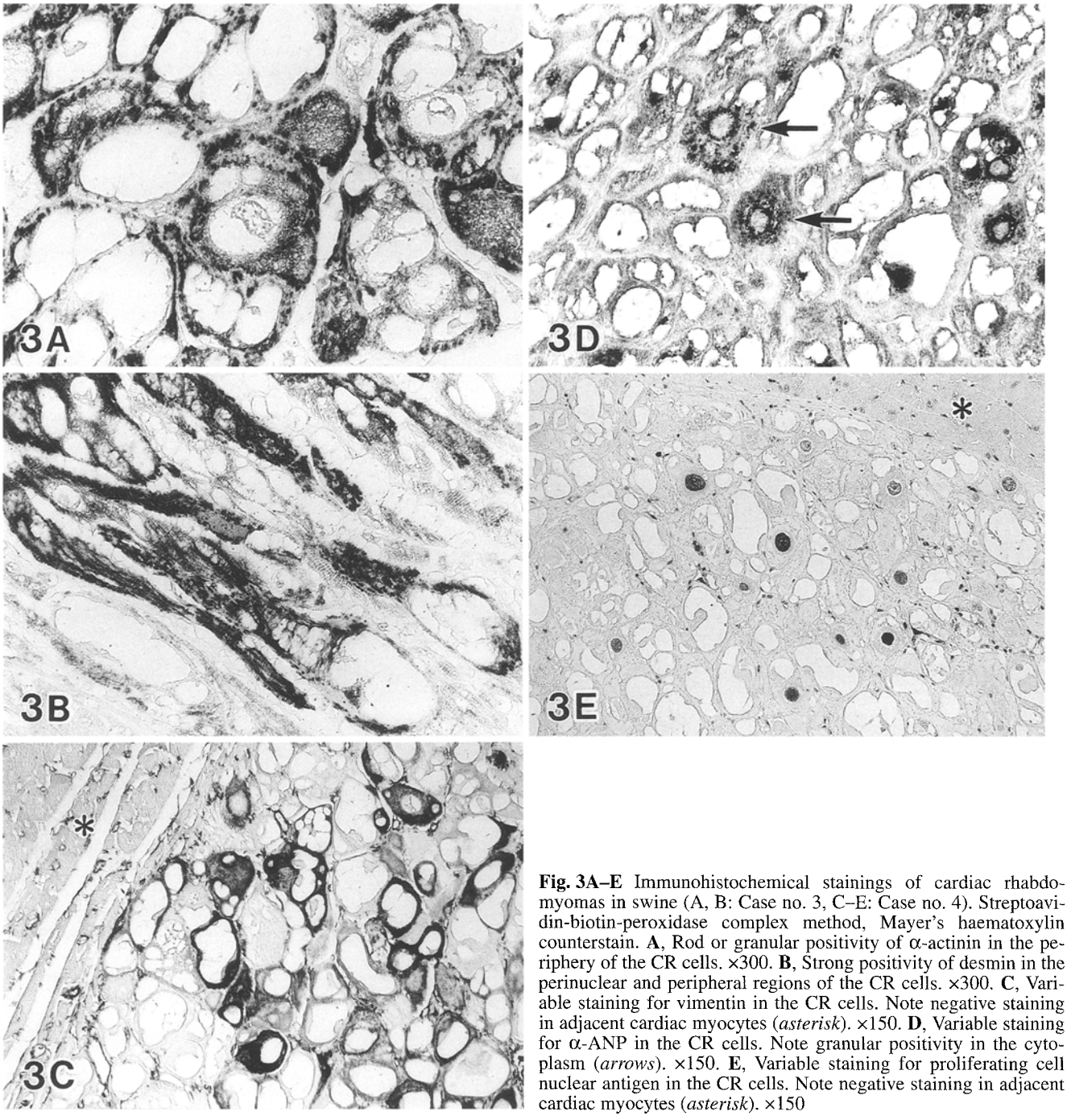

Fig. 3A–E Immunohistochemical stainings of cardiac rhabdomyomas in swine (A, B: Case no. 3, C–E: Case no. 4). Streptoavidin-biotin-peroxidase complex method, Mayer's haematoxylin counterstain. **A**, Rod or granular positivity of α-actinin in the periphery of the CR cells. ×300. **B**, Strong positivity of desmin in the perinuclear and peripheral regions of the CR cells. ×300. **C**, Variable staining for vimentin in the CR cells. Note negative staining in adjacent cardiac myocytes (*asterisk*). ×150. **D**, Variable staining for α-ANP in the CR cells. Note granular positivity in the cytoplasm (*arrows*). ×150. **E**, Variable staining for proliferating cell nuclear antigen in the CR cells. Note negative staining in adjacent cardiac myocytes (*asterisk*). ×150

maline bodies" seen in nemaline myopathy, were frequently observed (Fig. 4B) [12, 20]. Structures corresponding to "leptofibrils or zebra bodies" [12, 21], which were periodically arranged, electron-dense bands connected to one another by parallel arrays of thin filaments, were arranged either parallel to the cell surface or continuous with the Z bands (Fig. 4C). These Z band-related structures were increased in the vacuolated CR cells with myofibrillar degeneration (Figs. 4A, B). The sarcolemma of the CR cells, which was encircled by a well-defined basal lamina, was smooth. Tubular invaginations of the sarcolemma close to the Z-bands of the myofibrils were sometimes observed in longitudinal sections (Fig. 5A). The structures were also contact with the tubulovesicular elements in the myofibrils (figure not shown). These tubular structures closely resembled the T-system and sarcoplasmic reticulum of the cardiac muscle [19]. The CR cells were attached to each other by many desmosomes, which sometime showed a zigzag pattern (Fig. 5A) being similar to intercalated discs [19]. Variable amounts of electron-dense granules with a limiting membrane that associated with the Golgi apparatus in the perinuclear region were observed (Figs. 4A and 5B). The structures were similar to specific atrial gran-

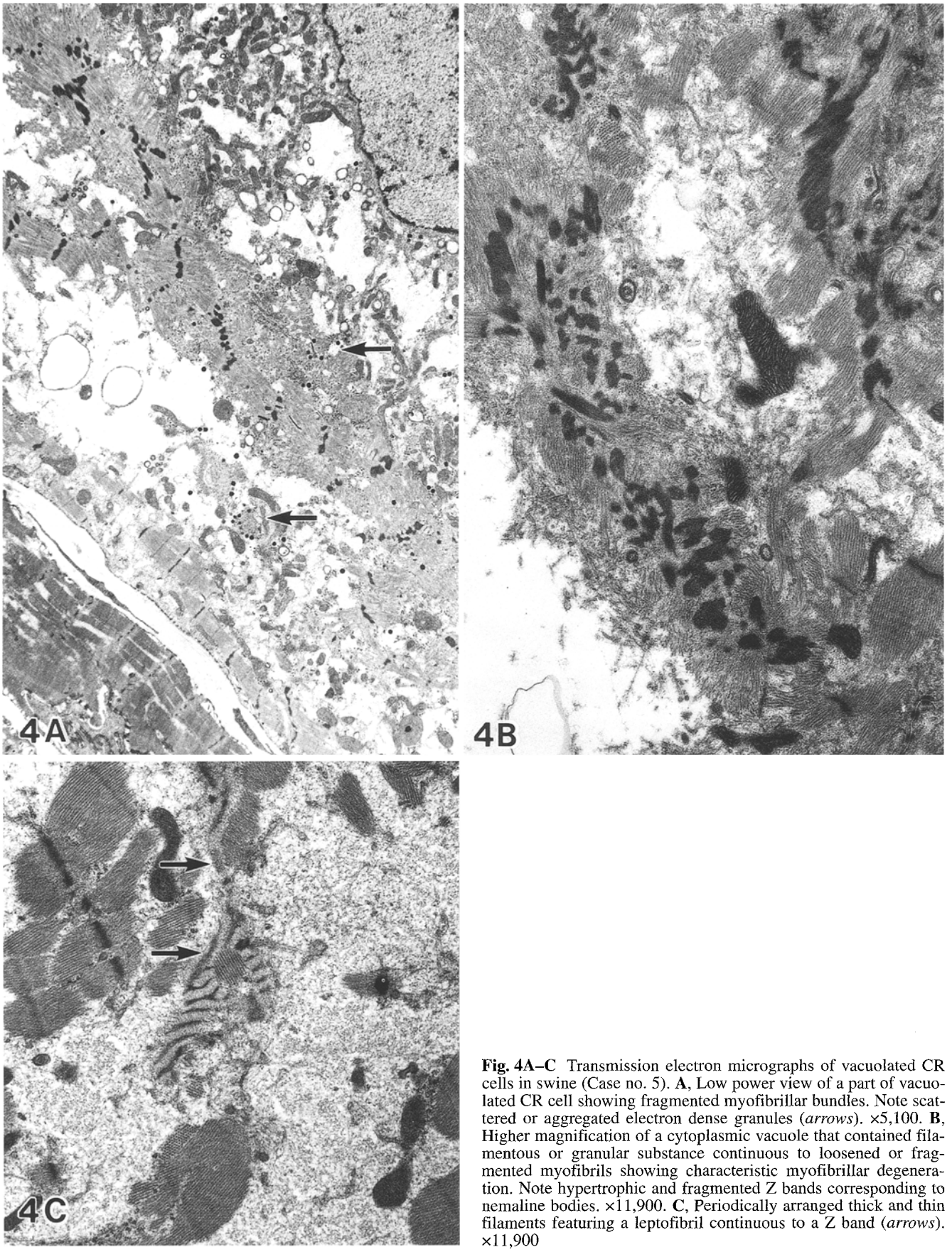

Fig. 4A–C Transmission electron micrographs of vacuolated CR cells in swine (Case no. 5). **A**, Low power view of a part of vacuolated CR cell showing fragmented myofibrillar bundles. Note scattered or aggregated electron dense granules (*arrows*). ×5,100. **B**, Higher magnification of a cytoplasmic vacuole that contained filamentous or granular substance continuous to loosened or fragmented myofibrils showing characteristic myofibrillar degeneration. Note hypertrophic and fragmented Z bands corresponding to nemaline bodies. ×11,900. **C**, Periodically arranged thick and thin filaments featuring a leptofibril continuous to a Z band (*arrows*). ×11,900

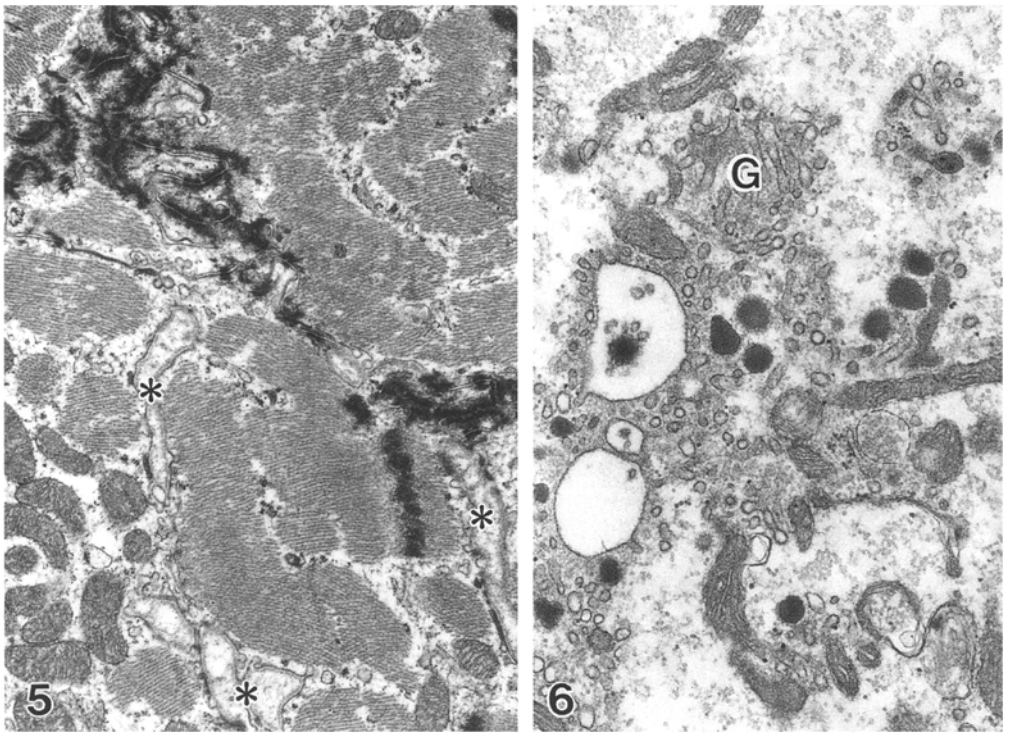

Fig. 5 Sarcolemmal invaginations corresponding to T-system (*asterisks*) (Case no. 5). Note many desmosomal attachments of intercalated discs showing a characteristic zigzag pattern. ×13,300

Fig. 6 Electron-dense granules associated with Golgi apparatus (*G*) similar to specific atrial granules (Case no. 5). ×26,600

ules [19]. Adjacent normal cardiac myocytes did not contain such granules. The CR cells had neither hypertrophic Z bands (corresponding to matchstick or jackstraw crystals) nor paracrystalline inclusions in the cytoplasm, and were not associated with satellite cells, these structures which frequently seen in extracardiac rhabdomyomas [33, 38].

Discussion

In this study, we found some new data, which suggest that CR in swine is a developmental failure of the perinatal cardiac tissues.

The macroscopic and microscopic features of the present neoplasms were essentially similar to those reported already for swine [5, 28, 31], but were partially like human CRs [2, 7]. Swine CR as in the present cases, are not found in the atrium [5, 28, 31]. However, in humans these lesions involve the atria [27]. Human CR is usually associated with a variety of congenital malformations and neoplasms, most commonly tuberous sclerosis [2, 11, 27]. These features are one of the reasons for assuming that CR is a hamartoma [2, 27]. Similar malformations have not been recognized in animals and none were seen in the present series.

The transitional feature (Fig. 2A, B) shows that swine CR mainly evolves from cardiac myocyte and rarely from Purkinje cell. Although a similar finding was described previously in human CR [34] and swine CR [5, 23, 28, 31], it was regarded as an earliest CR lesion [34], as a progressive expansion of CR lesion [31], or not emphasized [5, 23, 28].

To date, besides many clinical and histological studies of human CR, only one immunohistochemical [7] and some ultrastructural studies [6, 17, 18, 33, 34, 35, 38] have been reported. However, most of the studies were done with inadequately fixed materials. Therfore, CR has not been fully characterized yet even in humans.

To assess the immunophenotype of the CR cells, we used a broad range of antibodies reactive with striated muscle cells and their neoplasms [15, 25, 30, 37, 40, 41]. In contrast to our results in swine CRs (Table 3), only one report on human CRs [7] describes positivities for actin, desmin, myoglobin, and vimentin similar to those of adjacent cardiac myocytes. In the present study, the CR cells were positive for all muscular markers, α-ANP, and PCNA. Although the immunoreactivities for desmin, α-actinin, α-ANP, and vimentin were similar to those of Purkinje cells, positivities for these antigens are not specific for Purkinje cells. Moreover, increased positivities for desmin and vimentin have been reported in damaged or regenerating skeletal muscles and cardiac muscles [4, 12, 19, 20]. We confirmed the fact in swine myocarditis (Table 3). Variable staining for vimentin (Fig. 3C) and α-ANP (Fig. 3D) suggest that the CR cells are in various stages of differentiation even in the same nodule. Rod-like or granular intracytoplasmic positivity for α-actinin (Fig. 3B) may be compatible with the accumulation of hypertrophic Z-bands (nemaline bodies) (Fig. 4B), because α-actinin is mainly present in the Z bands of myofibrils [25].

Vimentin and cytokeratin are expressed during cardiac myogenesis, but gradually diminish during maturation and are finally absent in mature cardiac myocytes [30, 40]. Similarly, these expressions were observed in swine

cardiac myocytes, except vimentin positivity in Purkinje cells. Cytokeratin negativity of swine CR cells shows that they are not derived from fetal cardiac myocytes of early gestation, although human CR cells have been proposed to arise from primitive myocardial cells arrested in an early stage of differentiation from the ultrastructural findings [17, 18]. In an enzyme histochemical study, succinic dehydrogenase, which is normally demonstrable in embryonic and fetal cardiac tissues, was absent in CR cells [22]. This may further supports that CR does not originate from such cells. In a restricted number of human and murine rhabdomyosarcomas, the presence of neoplastic cells reactive for α-sr actin, neurofilament, and cytokeratin has been proved [41], but swine CR cells were negative for these antigens.

ANP, which has both natriuretic and diuretic activities consisted of three distinct forms (α-, β-, and γ-ANP), is stored in specific atrial granules [37]. The granular positivity for α-ANP in the CR cells may correspond to electron-dense granules associated with the Golgi apparatus (Figs. 4A, 5B). A rabbit antiserum against synthetic α-human ANP was reported to be immunoreactive with swine Purkinje cells [29, 37]. We used the same antiserum in this study, and found positivity of the CR cells like that of Purkinje cells. However, ANP is produced and secreted by ventricular myocytes in several human heart diseases [36], although the precise mechanism of this is unknown. We confirmed the fact in swine myocarditis (Table 3). In a human CR, α-ANP was shown in normal cardiac myocytes and the CR cells of both atria but not in those of both ventricles, and this was interpreted as further evidence that human CR is a hamartoma [35].

PCNA is a nuclear antigen expressed in all phases of the cell cycle except G0 [1]. It has been reported that adult human cardiac myocytes can express PCNA, and that this expression increases in hearts with hypertrophy or inflammation [1]. We confirmed its expression in swine myocarditis (Table 3). Then, interestingly, positive immunostaining for PCNA of the CR cells (Fig. 3E) with their enlarged cell size and no mitotic figures suggested that the CR cells did amitotic DNA renewal or repair caused by myofibrillar degeneration, and enlarged by increasing cell size rather than by proliferation as suggested previously [1, 5].

Some ultrastructural studies of human CRs have shown that the CR cells possess features of primitive cardiac myocytes [17, 18] or possess features of both cardiac myocytes and Purkinje cells [34], and therefore the histogenesis of this lesion could not be determined. However, the presence of the T-system (Fig. 5A), sarcoplasmic reticulum (figure not shown), and intercalated discs (Fig. 5A) are characteristics of mature cardiac myocytes that never seen in Purkinje cells [19]. The T-system develops in the late stages of cardiac cell development [6, 18]. In the present series as well as in previous reports, some features of Purkinje cells were detected such as scanty and unorganized myofibrils, a large amount of glycogen, ANP granules, and a large oval nucleus containing prominent nucleoli [19, 34]. However, these are not specific findings. Myofibrillar degeneration in the vacuolated CR cells is important for understanding the pathogenesis of CR with the transitional lesions at the periphery of some nodules (Fig. 2A, B). The exact reason for myofibrillar degeneration is undetermined, but it may be a feature of developmental failure of the cardiac tissues. Loss of myofibrils may impair the framework of the shape of the cardiac myocytes and consequently produce large cytoplasmic vacuoles. PAS-negative staining of the vacuoles (Fig. 1C) has been regarded as showing the loss of water-soluble glycogen during formalin fixation [3, 27]. Nemaline bodies and leptofibrils are not pathognomonic, because they are commonly observed in various muscular diseases including CR and in apparently normal hearts [12, 20, 21]. Their significance is obscure, but an increase of these structures near the sites of myofibrillar degeneration suggests they may be related to this lesion.

Recently, swine CRs were reported in five-day-old piglets [28]. With this report, our histological (transition from adjacent cardiac myocytes), immunohistochemical (negative staining for cytokeratin), and ultrastructural (presence of T-system, sarcoplasmic reticulum, and intercalated disc) findings suggest that swine CR may develop from perinatal cardiac tissues, which means cardiac tissues between a late gestational age and an early postnatal age.

CR is mostly believed to be hamartoma in origin. We essentially agree with this hypothesis with respect to its developmental failure. However, a hamartoma is a malformation that presents as a mass of disorganized but mature, fully-developed tissue indigenous to the particular site [10]. Taken with this definition and our study, we cannot interpret "cardiac rhabdomyoma" in swine as a mature specialized tissue, and thus, it does not belong to the entity of hamartoma. Instead of this, we more likely feel that congenital dysplasia with myofibrillar degeneration is a more appropriate term for this lesion. Dysplasia means epithelial or mesenchymal cells that have undergone proliferation and atypical cytological alterations involving cell size, shape, and organization [9, 32]. Dysplastic nature of CR has been previously suggested [13], and similar dysplastic myocardial lesions have been recognized in human hypertrophic cardiomyopathy and right ventricular dysplasia, and also in experimentally induced myocardial infarction of rats [14, 32]. Cardiac myocyte is a permanent cell, therefore CR cell may undergo dysplastic of hypertrophic adaptation rather than mitotic division when damaged or degenerated due to myofibrillar degeneration. The increase of cytoplasmic elements (glycogen, mitochondria, and atrial specific granules) and the varied immunopositivities of the CR cells (α-actinin, desmin, vimentin, α-ANP, and PCNA) can be interpreted as the reflection of reactive or regenerative changes.

In conclusion, our study shows that "cardiac rhabdomyoma" in swine may be a kind of congenital dysplasia of the perinatal cardiac tissues with myofibrillar degener-

ation, affecting mainly cardiac myocytes and rarely Purkinje cells.

Acknowledgements We thank Dr. K. Kangawa (Department of Biochemistry, Miyazaki Medical College) for providing polyclonal antibody against α-ANP. Portions of this paper were presented at the 44th Annual Meeting of the American College of Veterinary Pathologists, San Antonio, TX, 5–10 December 1993.

References

1. Arbustini E, Diegoli M, Grasso M, Fasani R, D'Armini A, Martinelli L, Goggi C, Campana C, Gavazzi A, Vigano' M (1993) Expression of proliferating cell markers in normal and diseased human hearts. Am J Cardiol 72:608–614
2. Batchelor TM, Maun ME (1945) Congenital glycogenic tumors of the heart. Arch Pathol 39:67–73
3. Beaird J, Mowry RW, Cunningham JA (1955) Congenital rhabdomyoma of the heart. Case report with histochemical study of tumor polysaccharide. Cancer 5:916–920
4. Bornemann A, Schmalbruch H (1992) Desmin and vimentin in regenerated muscles. Muscle Nerve 15:14–20
5. Bradley R, Wells GAH (1980) Ovine and porcine so-called cardiac rhabdomyoma (hamartoma). J Comp Pathol 90:551–558
6. Bruni C, Prioleau PG, Ivey HH, Nolan SP (1980) New fine structural features of cardiac rhabdomyoma: report of a case. Cancer 46:2068–2073
7. Burke AP, Virmani R (1991) Cardiac rhabdomyoma: a clinicopathlogic study. Mod Pathol 4:70–74
8. Cotran RS, Kumar V, Robbins SL (1989) Cellular injury and adaptation. In: Pathologic basis of disesase, 4th edn., WB Saunders, Philadelphia, pp 34–35
9. Cotran RS, Kumar V, Robbins SL (1989) Neoplasia. In: Pathologic basis of disease, 4th edn., WB Saunders, Philadelphia, p 243
10. Crome L (1954) The structural features of epiloia, with special reference to endocardial fibroelastosis. J Clin Pathol 7:137–140
11. Cullen MJ, Johnson MA, Mastaglia FL (1992) Pathological reactions of skeletal muscle. In: Mastaglia FL, Walton Lord (eds) Skeletal muscle pathology, 2nd edn., Churchill Livingstone, Edinburgh, pp 156–175
12. Davies MJ (1975) Myocardial tumours. In: Pomerance A, Davies MJ (eds) The pathology of the heart. Blackwell, Oxford, pp 420–424
13. Dusek J, Rona G, Kahn DS (1971) Healing process in the marginal zone of an experimental myocardial infarct. Findings in the surviving cardiac muscle cells. Am J Pathol 62:321–338
14. Enzinger FM, Weiss SW (1988) Immunohistochemistry of soft tissue lesions. In: Soft tissue tumors, 2nd edn., CV Mosby, St. Louis, pp 83–101
15. Farooki ZQ, Ross RD, Paridon SM, Humes RA, Karpawich PP, Pinsky WW (1991) Spontaneous regression of cardiac rhabdomyoma. Am J Cardiol 667:897–899
16. Fenoglio JJ, Diana DJ, Bowen TE, McAllister HA, Ferrans VJ (1977) Ultrastructure of a cardiac rhabdomyoma. Hum Pathol 8:700–706
17. Fenoglio JJ, McAllister HA, Ferrans VJ (1976) Cardiac rhabdomyoma: a clinicopathologic and electron microscopic study. Am J Cardiol 38:241–251
18. Ferrans VJ, Rodriguez ER (1991) Ultrastructure of the normal heart. In: Silver MD (ed) Cardiovascular pathology, 2nd edn., vol 1, Churchill Livingstone, Edinburgh, pp 43–101
19. Ghadially FN (1988) Intracytoplasmic filaments. In: Ultrastructural pathology of the cell and matrix, 3rd edn., vol 2, Butterworths, London, pp 848–853
20. Ghadially FN (1988) Cytoplasmic matrix and its inclusions. In: Ultrastructural pathology of the cell and matrix, 3rd edn., vol 2, Butterworths, London, pp 1002–1003
21. Golding R, Reed G (1967) Rhabdomyoma of the heart. Two unusual clinical presentations. N Engl J Med 276:957–959
22. Hadlow WJ (1962) Diseases of skeletal muscle. In: Innes JRM, Saunders LZ (eds) Comparative neuropathology. Academic Press, New York, pp 224–225
23. Hulland TJ (1990) Tumors of striated muscle. In: Molton JE (ed) Tumors in domestic animals, 3rd edn. University of California Press, Berkeley, pp 88–101
24. Jockusch BM, Veldman H, Griffiths GW, van Oost BA, Jennekens FGI (1980) Immunofluorescence microscopy of a myopathy. α-actinin is a major constituent of Nemaline rods. Exp Cell Res 127:409–420
25. McAllister HA, Fenoglio JJ (1978) Primary tumors and cysts of the heart and pericardium. In: Tumors of the cardiovascular system, atlas of tumor pathology, 2nd series, fasc. 3. Armed Forces Institute of Pathology, Washington DC, pp 25–31
26. McEwen BJE (1994) Congenital cardiac rhabdomyomas in red wattle pigs. Can Vet J 35:48–49
27. Miyata A, Kangawa K, Toshimori T, Hatoh T, Matsuo H (1985) Molecular forms of atrial natriuretic polypeptides in mammalian tissue and plasma. Biochem Biophys Res Commun 129:248–255
28. Van Muijen GNP, Ruiter DJ, Warnaar SO (1987) Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 57:359–369
29. Omar AR (1969) Congenital cardiac rhabdomyoma in a pig. Vet Pathol 6:469–474
30. Silver MM, Silver MD (1991) Cardiomyopathies. In: Cardiovascular pathology, 2nd edn., vol 1, Churchill Livingstone, Edinburgh, pp 743–809
31. Silverman JF, Kay S, Chang CH (1978) Ultrastructural comparison between skeletal muscle and cardiac rhabdomyomas. Cancer 42:189–193
32. Silverman JF, Kay S, McCue CM, Lower RR, Brough AJ, Chang CH (1976) Rhabdomyoma of the heart. Ultrastructural study of three cases. Lab Invest 35:596–606
33. Takatoh H, Iwamoto H, Ikezu M, Katoh N, Kaneko H, Ishikawa H, Kamoi K (1988) Cardiac rhabdomyoma. A case report with reference to atrial natriuretic peptide. Acta Pathol Jpn 38:95–104
34. Takemura G, Fujiwara H, Horike K, Mukoyama M, Saito Y, Nakao K, Matsuda M, Kawamura A, Ishida M, Kida M, Uegaito T, Tanaka M, Matsumori A, Fujiwara Y, Fujiwara T, Imura H, Kawai C (1989) Ventricular expression of atrial natriuretic polypeptide and its relations with hemodynamics and histology in dilated human hearts. Immunohistochemical study of the endomyocardial biopsy specimens. Circulation 80:1137–1147
35. Toshimori H, Toshimori K, Okura C, Matsuo H, Matsukura S (1988) Immunohistochemical identification of Purkinje fibers and transitional cells in a terminal portion of the impulse-conducting system of porcine heart. Cell Tissue Res 253:47–53
36. Trillo AA, Holleman IL, White JT (1978) Presence of satellite cells in a cardiac rhabdomyoma. Histopathology 2:215–223
37. Tsukada T, McNutt MA, Ross R, Gown AM (1987) HHF35, a muscle actin-specific monoclonal antibody. II. Reactivity in normal, reactive, and neoplastic human tissues. Am J Pathol 127:389–402
38. Velez C, Muros AE, Aranega JE, Fernandez JE, Gonzalez FJ, Alvarez L, Aranega A (1990) Coexpression of intermediate filament proteins in the chick embryo heart. Acta Anat 139:226–233
39. Vos JH, Borst GHA, de las Mulas JM, Ramaekers FCS, van Mil FN, Molenbeek RF, Ivanyi D, van den Ingh TSGAM (1993) Rhabdomyosarcomas in young pigs in a swine breeding farm. A morphologic and immunohistochemical study. Vet Pathol 30:271–279