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Proliferative Potential of Cardiomyocytes in Hypertrophic Cardiomyopathy: Correlation with Myocardial Remodeling

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Proliferating Ki-67$^+$ cardiomyocytes were detected in the interventricular septum myocardium of adult patients with hypertrophic cardiomyopathy. In the same patients, the severity of hypertrophy and the degree of cardiomyocyte differentiation were assessed by the content of myofibrils, ultrastructural morphology, and the pattern of connexin 43-containing gap junction distribution. Adult Ki-67$^+$ cardiomyocytes containing sarcomeric α-actin (sarc α-act$^+$) in the sarcoplasm (diameter 23.9±6.9 μ) were detected in the myocardium of patients with hypertrophic cardiomyopathy; their relative content varied from 2 to 3084 cells per 1 million cardiomyocytes. Small early differentiating Ki-67$^+$/sarc α-act$^+$ cardiomyocytes with a thin cytoplasm layer (diameter 5.9±1.7 μ) constituted from 3 to 2262 cells per 1 million cardiomyocytes. These cells were found in the myocardium with the most pronounced structural changes: hypertrophy of cardiomyocytes with signs of their partial dedifferentiation.

Key Words: *hypertrophic cardiomyopathy; myocardium of the interventricular septum; proliferative activity of cardiomyocytes; connexin 43; dedifferentiated cardiomyocytes*

In patients with hypertrophic cardiomyopathy (HCM), critical hypertrophy of the interventricular septum (IVS) requiring surgical intervention is primarily determined by hypertrophy of IVS cardiomyocytes. IVS enlargement disturbs systolic and diastolic functions of the left ventricle (LV), leads to the formation of dynamic pressure gradient in the LV, myocardial ischemia, affects electrophysiological properties associated with increased risk of arrhythmias, and can be the cause of sudden death [2]. Reactivation of proliferative processes in cardiomyocytes can also contribute to the development of IVS hypertrophy. The possibility of cardiomyocytes proliferation in mature myocardium was convincingly demonstrated in experimental and clinical studies [9,14,23,24,40,43,44]. The combination of hypertrophy and hyperplasia was previously described in cardiomyocyte hypertrophy caused by increased workload [43,44] and under some pathological conditions, in particular, in the myocardium of patients with HCM [6], stenosis of the aortic valve [40], in the LV myocardium of patients with coronary haert disease (CHD) [9,23]. The number of proliferating cardiomyocytes in patients with different cardiovascular disease varies in a side range from 41 [9] to 3400 cells per 1 million cardiomyocytes [14]. This considerable discrepancy in quantitative data can be related to different experimental methods; however, activation of proliferative processes was noted in all cases.

Hypertrophy and reproduction capacity of cardiomyocytes are inextricably linked with the degree of myocardium differentiation. In our previous studies, we described the combination of cardiomyocyte hypertrophy with the appearance of signs of their partial dedifferentiation: reduced content of myofibrils and reactivation of natriuretic peptide synthesis; in addition, activation of resident stem cells, cardiomyocyte precursors, was found [4].

The purpose of this study was to assess the proliferative potential of the IVS myocardium and to determine its relationship with structural rearrangement of hypertrophic cardiomyocytes in HCM patients.

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MATERIALS AND METHODS

We studied biopsy specimens of IVS myocardium from 35 adult patients with obstructive HCM obtained during surgical myectomy from the right ventricle (Table 1). Proliferating cardiomyocytes were detected in the IVS myocardium fragments fixed in 4% neutral paraformaldehyde (Immunofix, Bio-Optica) and embedded in paraffin.

For double immunohistochemical staining, the paraffin sections were incubated with a mixture (1:1) of primary antibodies to proliferation marker Ki-67 (Abcam) and sarcomeric α-actin (sarc α-act; Abcam) and then with a mixture (1:1) of second antibodies labeled with fluorochromes Alexa 488 and Alexa 546 (Invitrogen). Nuclei were poststained with DAPI (Sigma). Positive immunohistochemical reaction for Ki-67 indicated released from G_0 phase cycling myocytes; differentiation of cardiomyocytes was confirmed by positive reaction for sarc α-act. The preparations were examined under a Leica TCS SPE confocal microscope (Carl Zeiss). Ki-67$^+$/sarc α-act$^+$ cardiomyocytes were counted. The section area and the density of CMC were determined. The proportion of Ki-67$^+$ cardiomyocytes per 1 million myocytes was determined and expressed as the median and the maximum and minimum values.

For morphometric analysis (on semithin sections) and electron microscopy, the specimens were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M PBS (pH 7.4), postfixed in 1.5% OsO_4, dehydrated, and embedded in araldite. The diameter of cardiomyocytes and their nuclei were measured on Schiff-stained semithin sections poststained with methylene blue. Myofibril loss in cardiomyocytes was scored by a 4-point scale as follows: free zones of the sarcoplasm filled with glycogen occupy <10% cell cross-section area (0), 10-50% (1), 50% (2), and >50% (3). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a Philips CM100 electron microscope.

Connexin 43 (Cx43), a gap junction protein in the intercalated discs, was detected in paraffin sections of IVS myocardium by immunohistochemical staining with specific monoclonal antibodies (Sigma). The length and diameter of at least 50 cardiomyocytes were measured under a light microscope on longitudinal sections through the nucleus and intercalated discs. In the same cells, the length of Cx43$^+$ sarcolemma segments on the lateral sides of cardiomyocytes was measured and expressed in percent of the doubled myocyte length (relative length of Cx43$^+$ gap junctions).

The data on the contents of proliferating Ki-67$^+$/sarc α-act$^+$ cardiomyocytes were compared with the characteristics of the IVS myocardium and parameters of clinical examination of the patients and analyzed using non-parametric Spearman's correlation coefficient at a significance level of $p<0.05$.

RESULTS

Proliferative activity of IVS cardiomyocytes. Immunoconfocal microscopy revealed two populations of proliferating Ki-67$^+$/sarc α-act$^+$ cardiomyocytes in IVS myocardium: highly differentiated adult and small developing cardiomyocytes; the appearance of these subsets was interrelated (r=0.62; p=0.00006).

Adult Ki-67$^+$/sarc α-act$^+$ cells with a diameter of 23.9±6.9 μ filled with myofibrils were found in the myocardium of all patients with HCM (Fig. 1, *a*); their content varied considerably: from 2 to 3084 (Me 112) cells/million cardiomyocytes (Fig. 1, *c*) and correlated with the severity of hypertrophy (thickness) of IVS (r=0.34; p=0.044) (Fig. 1, *d*).

The presence of mature proliferating Ki-67$^+$/sarc α-actin$^+$ cardiomyocytes in normal rat myocardium has been described in a previous report, and their content increased with increasing load intensity and duration [44]. In the LV myocardium of mice with experimentally-induced cardiomyocyte hypertrophy due to pressure overload, 0.1±0.02% Ki-7$^+$/sarc α-actin$^+$cells were found, while in the control group, these cells were absent [24]. It should be noted that both hypertrophy and hyperplasia induced by transient load were adaptive in nature and the size of cardiomyocytes and the content of Ki-67$^+$ myocytes returned to the initial level after a similar period of detraining [43].

Considerable activation of proliferative processes was observed in the myocardium of patients with cardiovascular pathologies. For instance, about 3400 adult Ki-67$^+$/sarc α-actin$^+$ cells/million cardiomyocytes were detected in LV of patients with postinfarction ischemic and idiopathic dilated cardiomyopathy, which significantly exceeded the corresponding value in patients without cardiovascular pathologies (~300-1600 cells/million cardiomyocytes) [14]. In hypertrophied hearts of patients with aortic stenosis, the content of Ki-

TABLE 1. Clinical Characteristics of Patients with HCM (N=35)

Parameter	$M\pm m$	Range
Age, years	37.8±13.1	17-61
IVS thickness, mm	24.6±5.3	12-35
End-systolic LV volume, ml	24±10	9-47
End-diastolic LV volume, ml	81±28	32-139
Ejection fraction, %	74.0±7.5	56-91
LV outlet pressure gradient, mm Hg	96±35	42-205

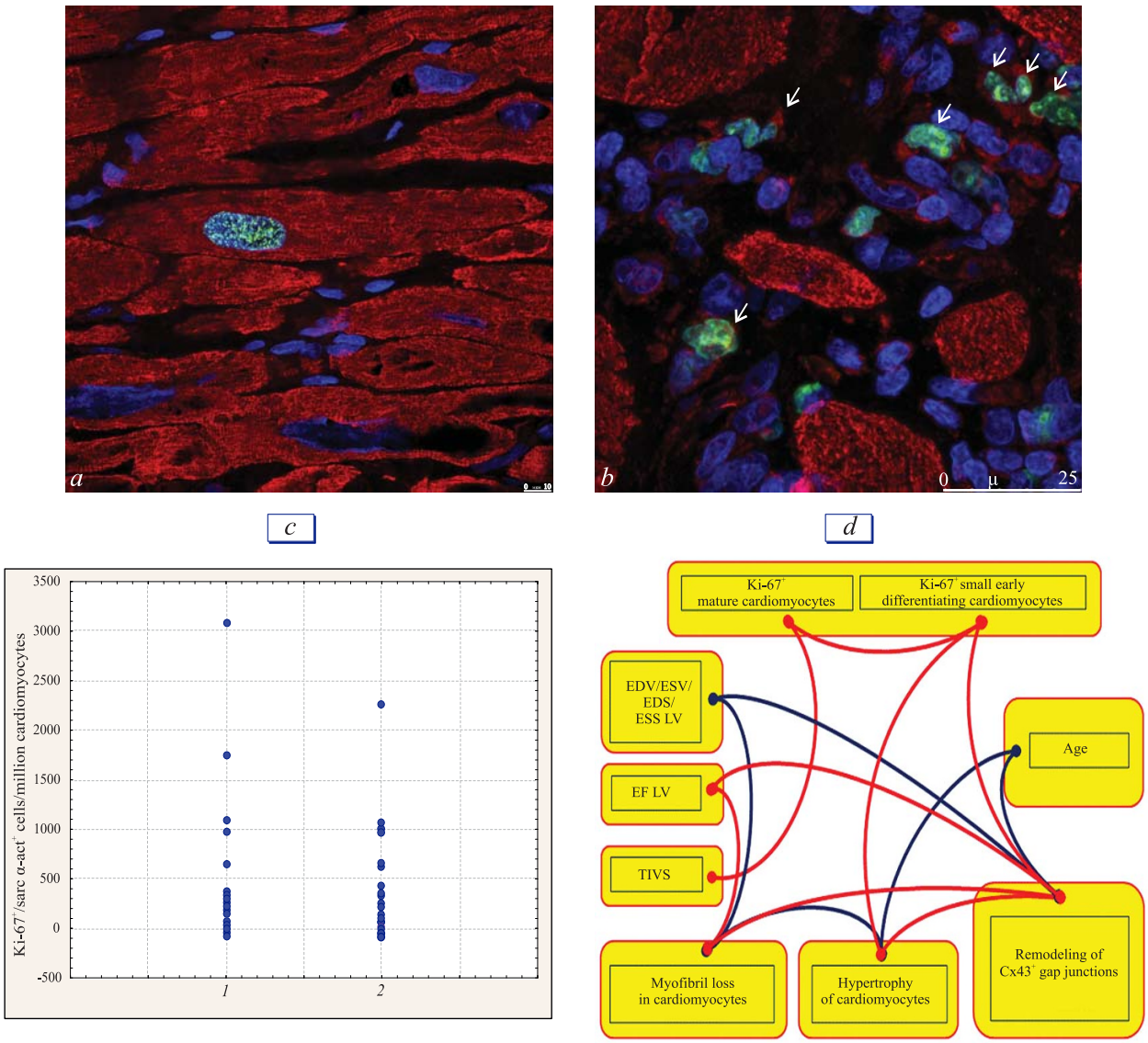

Fig. 1. Proliferating (Ki-67$^+$/sarc α-act$^+$) cardiomyocytes in IVS myocardium of patients with HCM. Immunofocal microscopy (*a*, *b*), Alexa 488 (Ki-67), Alexa 546 (sarc α-actin). Nuclei were poststained with DAPI (blue). *a*) Mature differentiated cardiomyocytes (diameter 23.9±6.9 μ) contain Ki-67$^+$ nuclei and myofibrils with sarc α-actin in the sarcoplasm; *b*) small early differentiating cardiomyocyte precursors (diameter 5.9±1.7 μ) with Ki-67$^+$ nuclei surrounded by a thin layer of cytoplasm containing sarc α-actin (arrows). *c*) Distribution of Ki-67$^+$/sarc α-act$^+$ cardiomyocytes; mature (*1*) and small low differentiated cardiomyocytes (*2*) in IVS myocardium in patients with HCM. *d*) Relationship of cardiomyocyte proliferative activity with parameters characterizing structural remodeling of IVS myocardium in patients with HCM (Spearmen's correlation coefficient: $r>0$ red arrow, $r<0$ blue arrow, $p<0.05$). EDV and ESV: end-diastolic and end-systolic volumes of the LV, respectively; EDS and ESS: end-diastolic and end-systolic size of the LV, respectively; EF: ejection fraction; TIVS: IVS thickness.

67$^+$cells was 2100±740 cells/million cardiomyocytes, which 150-180-fold exceeded their content in the control (15±5 cells/million of cardiomyocytes) [40]. In the LV myocardium of CHD patients, the number of adult Ki-67$^+$/sarc α-actin$^+$ cells on days 4-12 after myocardial infarction increased by 28-84% and attained ~41 cells/million cardiomyocytes in the periinfarction area and ~13 cells/million of cardiomyocytes in the distant myocardium [9].

The maximum content of adult Ki-67$^+$/sarc α-actin$^+$ cardiomyocytes in the periinfarction myocardium (11.61±6.94%) was observed during the second week after myocardial infarction (*vs*. 0.334±0.75% at the earlier and 2.66±3.04% at the late terms). Activation of proliferative processes led to an increase in the number of endomitoses followed (on postinfarction days 14-21) by an increase in the content high-ploidy (8c) cardiomyocytes in periinfarction myocardium [23]. Increased ploidy of IVS cardiomyocytes in adult patients with HCM was previously reported [5,6,12,22]; DNA content was equal to 4c [12] (according to other reports, this value varied from 2.9-13.5c [5] to 3.5-17.7c

[6]). It is now accepted that increased ploidy provides a reserve for additional compensatory cardiomyocyte growth under conditions of pathological overload. However, in patients with HCM, the compensatory hypertrophy of IVS cardiomyocytes apparently exacerbates LV obstruction, which leads to deterioration of their clinical status.

Another population of Ki-67$^+$/sarc α-act$^+$ cells identified in our study were small early differentiating cardiomyocytes (mean diameter of 5.9±1.7 μ). These myocytes with a narrow cytoplasm layer containing sarcomeric α-actin were usually located in the interstitium one by one or formed small groups (Fig. 1, *b*). They were detected in 32 of 35 patients with HCM and their content varied from 3 to 2262 (Me 103) cells/million cardiomyocytes (Fig. 1, *c*). Similar clusters of small Ki-67$^+$ cardiomyocytes precursors were described in the LV myocardium of patients with aortic stenosis [40]. Apparently, these myocytes partially represented the population of c-kit$^+$/sarc α-actin$^+$ resident cardiomyocyte precursors identified previously by us in patients with HCM [4].

Morphological analysis of IVS myocardium in patients with HCM. Morphological analysis of IVS myocardium revealed considerable variability of the degree of cardiomyocytes hypertrophy, the cell diameter ranged from 8.8 to 33.8 μ (mean 23.2±4.8 μ) (Fig. 2, *a*, *b*). Cardiomyocyte diameter increased in parallel with their elongation (r=0.64; p=0.001). Cardiomyocytes usually densely packed with myofibrils (Fig. 2, *c*) contained nuclei with a diameter of 2.4-7.8 μ (mean 5.2±0.9 μ). At the ultrastructural level, most cardiomyocytes demonstrated features typical of hypertrophic cells with high synthetic activity: large nuclei contained nucleoli, sarcoplasm was filled with myofibrils, perinuclear area contained structures of the Golgi complex, cisterns of the granular endoplasmic reticulum, and widened T-system channels (Fig. 2, *d-f*). The most pronounced cardiomyocyte hypertrophy was observed in younger patients (r=-0.42; p=0.013) (Fig. 1, *d*; Fig. 2, *g*).

In most cardiomyocytes, myofibrils filled the main part of sarcoplasm, but in 26 of 35 patients (74.3%), myocytes with reduced content of myofibrils in perinuclear area of the sarcoplasm were found. Under a light microscope, these areas in preparations stained with hematoxylin and eosin looked optically empty (Fig. 3, *a*), while on semithin sections, they corresponded to accumulations of glycogen granules (Fig. 3, *b*). At the ultrastructural level, the cardiomyocyte sarcoplasm contained numerous mitochondria, cisterns of the granular endoplasmic reticulum, tubules and vesicles of the Golgi complex, lipofuscin granules (Fig. 3), as well as structures typical ofundifferentiated cardiomyocytes, such as myofibril complexes with ribosomes (Fig. 3, *g*); single cells contained centrioles (Fig. 3, *e*).

The appearance of myofibril-free zones in the cardiomyocyte sarcoplasm is untypical of HCM. These structural changes in cardiomyocytes is typically observed in patients with myocardial ischemia [8,20], in atrial cardiomyocytes of patients with atrial fibrillation [39] and valvular heart diseases [29]. Partial disassembly of myofibrils in these cardiomyocytes was accompanied not only by reduced expression of contractile proteins of mature cardiomyocytes (α-titin, α-actinin, and cardiotin), but also re-expression of embryonic cardiomyocyte proteins (vascular smooth muscle actin and β-myosin heavy chain) [8,29]. It is believed that this is a manifestation of adaptive response of myocytes exposed to chronic energy deficiency due to reduced blood flow or increased functional load. Under these conditions, mature differentiated cardiomyocytes partially lost some specialized structures (myofibrils) and acquired features of undifferentiated cardiomyocytes [3].

The possibility of cardiomyocyte dedifferentiation up to re-entry into the cell cycle is now actively discussed. It is shown that mature differentiated cardiomyocytes isolated from human atria during culturing under certain *in vitro* conditions undergo dedifferentiation accompanied by changes in cardiomyocyte size and shape, as well as localization of sarcomeric proteins [29]. For instance, the distribution of α-titin and α-actinin in *in vitro* dedifferentiated cardiomyocytes was similar to those in cardiomyocytes with zones of partial myofibril loss *in vivo* [29]. Similar experiment on rodent cardiomyocytes showed that cells cultured in a medium enriched with mitogens lost their contractile structures, changed their electrophysiological characteristics and, starting from day 2 in culture, reentered the mitotic cycle and expressed markers of proliferation (Ki-67) and stem cells (C-kit) [45]. Thus, the possibility of remodeling and dedifferentiation of mature cardiomyocytes in response to modification of the environmental conditions was demonstrated.

The presence of cardiomyocytes with myofibril loss in the myocardium of patients with HCM can be explained by increased oxygen demand of hypertrophied muscle fibers. Moreover, the pathology of microcirculatory bed with reduced density of capillaries promotes the development of energy deficiency in the myocardium of these patients. Clinical manifestations of myocardial ischemia are observed in 30% patients with HCM [1]. We found that partial loss of myofibrils involving 10-50% and more cardiomyocyte sarcoplasm was more often detected in the myocardium of patients with most severe clinical condition: reduced end-diastolic volume of LV (r=-0.51; p=0.002) and increased LV ejection frac-

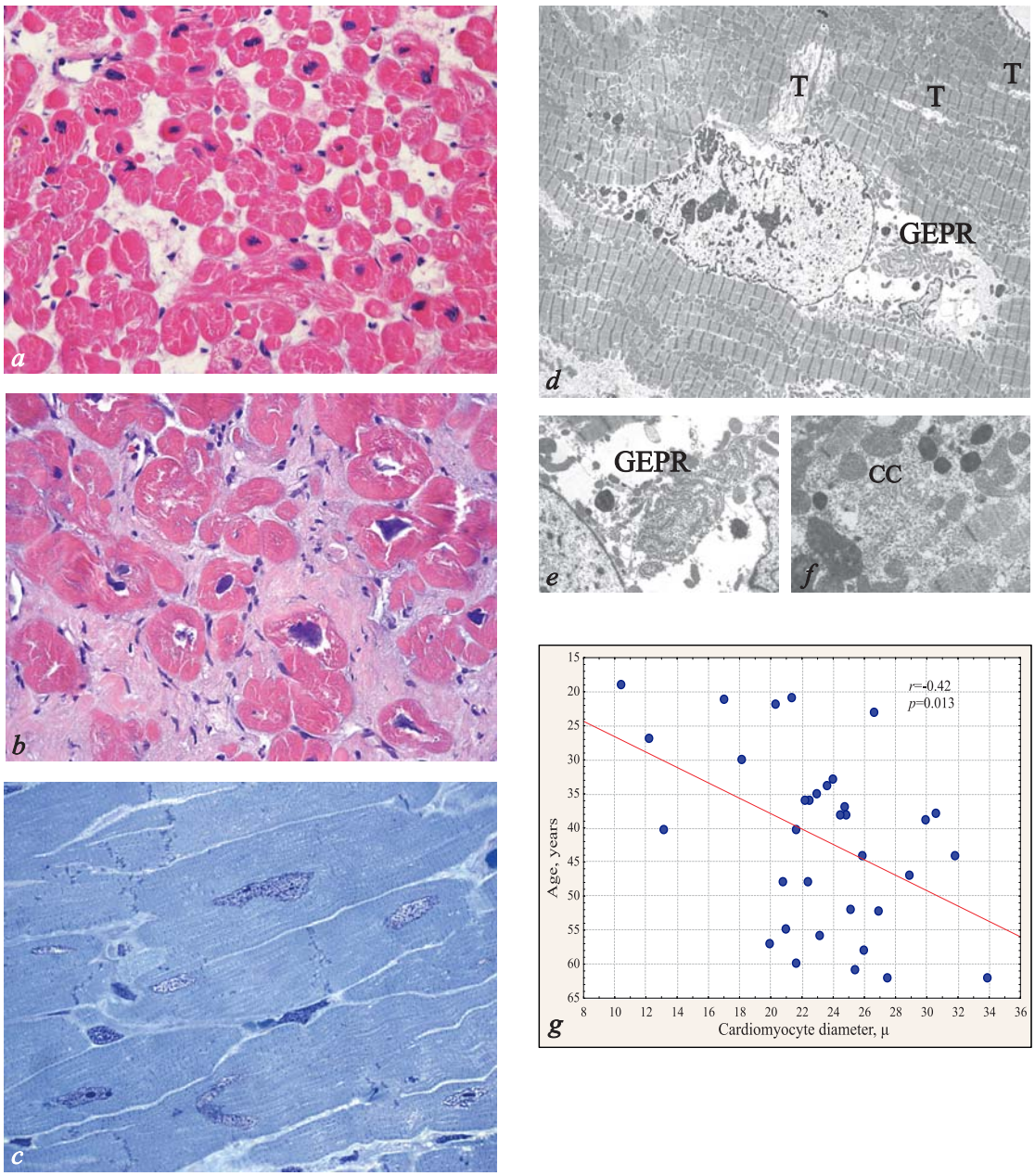

Fig. 2. Hypertrophy of IVS cardiomyocytes in patients with HCM. *a*) Cells with low degree of hypertrophy (mean diameter 20.9±3.7 μ); patient with HCM, 58 years; *b*) Cells with high degree of hypertrophy (mean diameter 36.1±11.6 μ); patient with HCM, 35 years. Hematoxylin and eosin staining, ×200. *c*) Hypertrophied cardiomyocytes, sarcoplasm packed with myofibrils. PAS-methylene blue stained semithin sections, ×100. *d-f*) Ultrastructure of a hypertrophied cardiomyocyte: nucleus with several nucleoli, invagination of the nuclear membrane, well-developed granular endoplasmic reticulum (GEPR) in the perinuclear sarcoplasm (*d*, *e*), mitochondria, lipofuscin granules, cisterns and vesicles of the Golgi complex (GC) (*d*, *f*). Sarcoplasm is packed with myofibrils, widened T-system channels (T). ×1600 (*d*); ×6500 (*e*), ×11,000 (*f*); *g*) inverse correlation between the increase in cardiomyocyte diameter and the age of patients with HCM (r=-0.42; p=0.013).

tion (r=0.47; p=0.004) according to ultrasound examination (Fig. 1, *d*; Fig. 3, *f*, *g*). Remodeling with myofibril loss was usually observed in small cardiomyocytes with exhausted resources for hypertrophic growth (Fig. 1, *d*): elongated cells (r=0.61; p=0.002) with small diameter (r=-0.62; p=0.0004). The presence of these cardiomyocytes in IVS myocardium of patients with HCM could serve as a marker of early structural changes indicating transition from compensated myocardial hypertrophy to a decompensated state without clinical manifestations.

Changes in the cardiomyocyte structure were accompanied by redistribution of $Cx43^+$ gap junctions responsible for electrical coupling of neighboring cardiomyocytes over the sarcolemma surface. In mature cardiomyocytes of the IVS myocardium, $Cx43^+$ gap

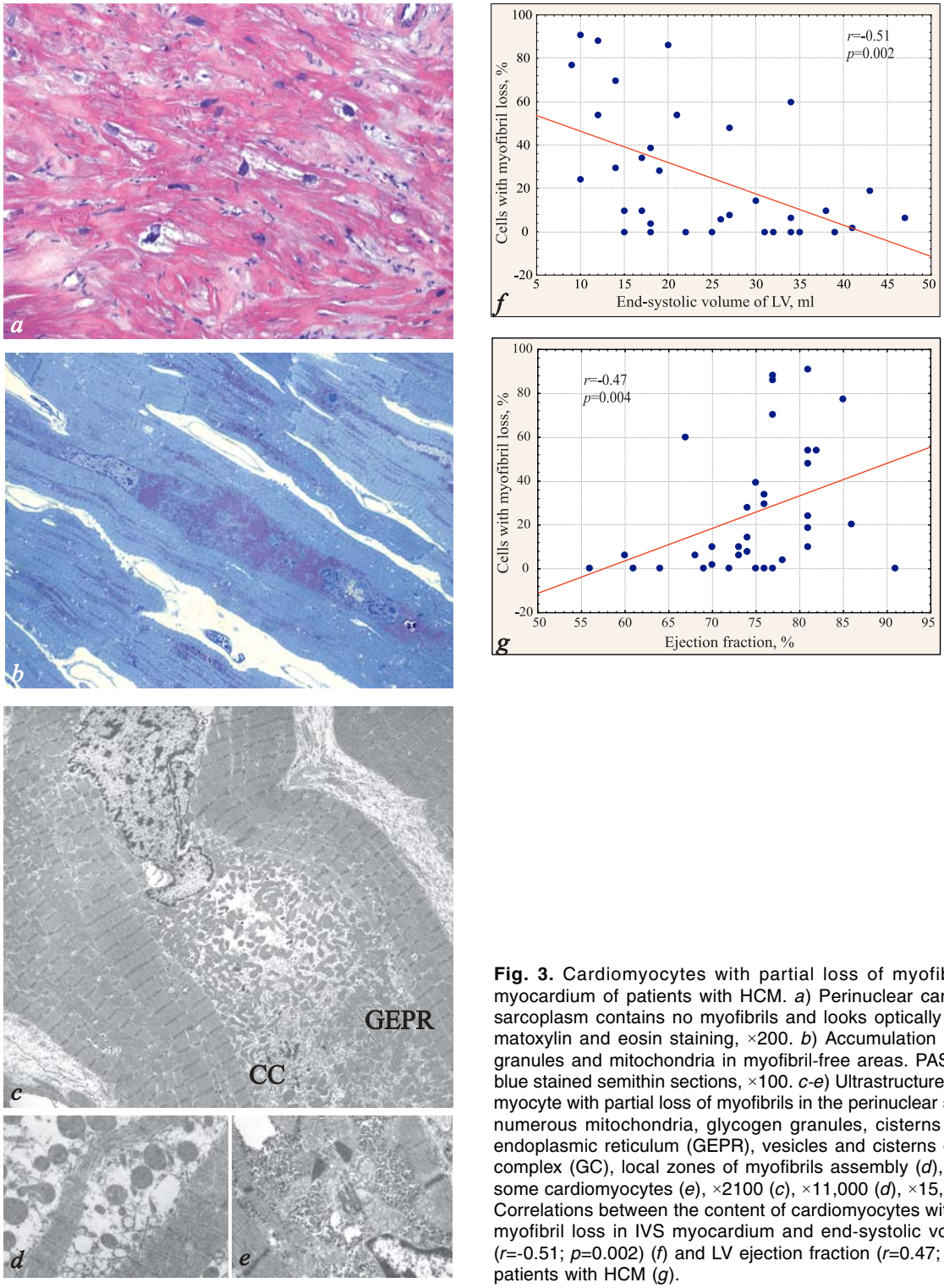

Fig. 3. Cardiomyocytes with partial loss of myofibrils in IVS myocardium of patients with HCM. *a*) Perinuclear cardiomyocyte sarcoplasm contains no myofibrils and looks optically empty. Hematoxylin and eosin staining, ×200. *b*) Accumulation of glycogen granules and mitochondria in myofibril-free areas. PAS-methylene blue stained semithin sections, ×100. *c-e*) Ultrastructure of a cardiomyocyte with partial loss of myofibrils in the perinuclear sarcoplasm: numerous mitochondria, glycogen granules, cisterns of granular endoplasmic reticulum (GEPR), vesicles and cisterns of the Golgi complex (GC), local zones of myofibrils assembly (*d*), centriole in some cardiomyocytes (*e*), ×2100 (*c*), ×11,000 (*d*), ×15,000 (*e*). *f-g*) Correlations between the content of cardiomyocytes with moderate myofibril loss in IVS myocardium and end-systolic volume of LV (r=-0.51; p=0.002) (*f*) and LV ejection fraction (r=0.47; p=0.004) in patients with HCM (*g*).

junctions were predominantly located on intercalated disk sides (Fig. 4, *a*) parallel to the cardiomyocyte long axis. Membranes of the adjacent myocytes in these sites maximally close approach each other, so that the gap between them is 2-4 nm (Fig. 4, *a*, insert). However, in some cardiomyocytes, most often, in cardiomyocytes with partial loss of myofibrils, $Cx43^+$ gap junctions were seen not only in the intercalated disks, but also on the lateral sides of the cells (Fig. 4, *b*). The length of these lateral contacts varied from 5.2 to 59.6 μ and constituted 4-28% of the doubled cardiomyocyte length. Remodeling of gap junctions was most likely related to the formation of additional intercalated disc to ensure "side to side" contacts between

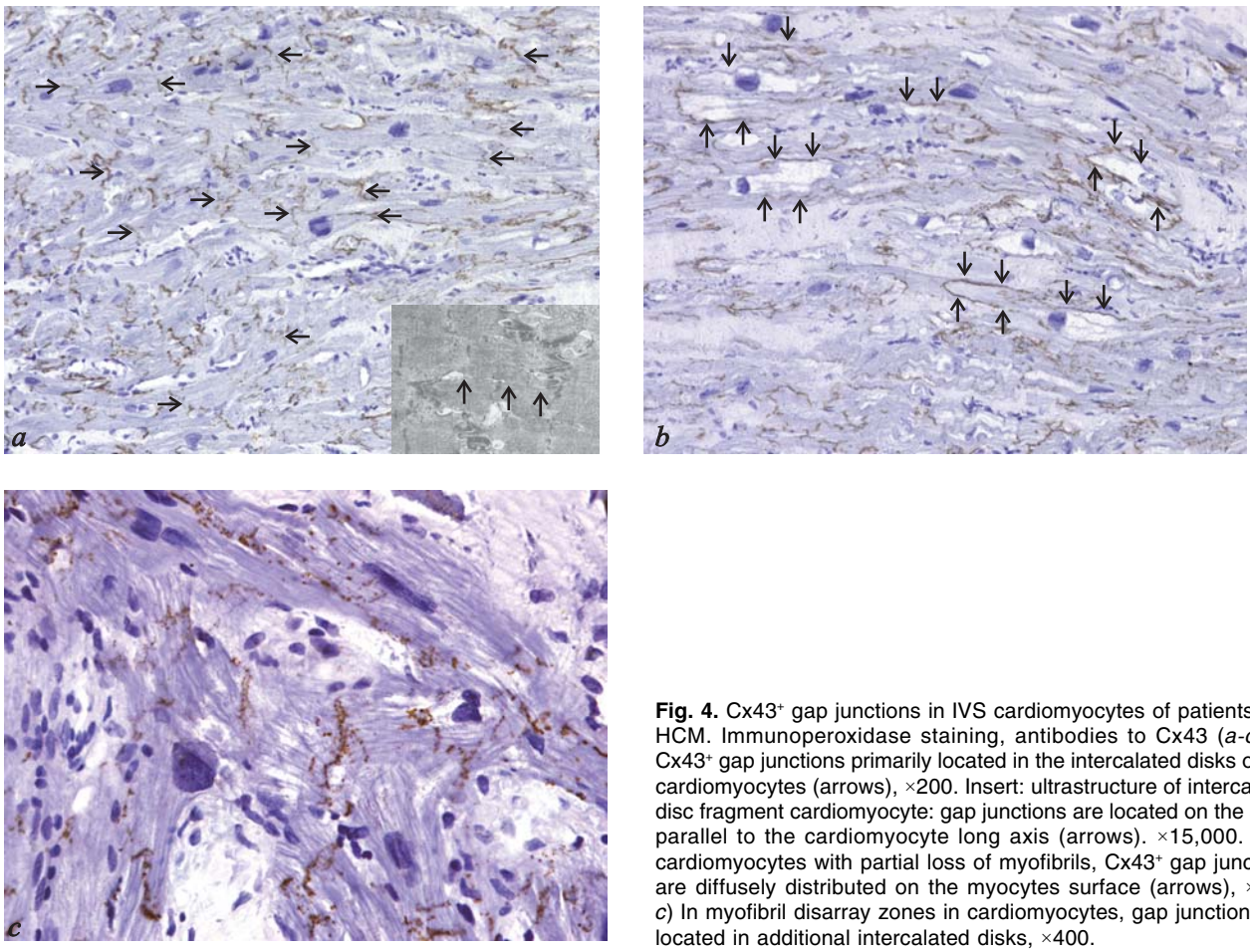

Fig. 4. Cx43$^+$ gap junctions in IVS cardiomyocytes of patients with HCM. Immunoperoxidase staining, antibodies to Cx43 (*a-c*). *a*) Cx43$^+$ gap junctions primarily located in the intercalated disks of IVS cardiomyocytes (arrows), ×200. Insert: ultrastructure of intercalated disc fragment cardiomyocyte: gap junctions are located on the sides parallel to the cardiomyocyte long axis (arrows). ×15,000. *b*) In cardiomyocytes with partial loss of myofibrils, Cx43$^+$ gap junctions are diffusely distributed on the myocytes surface (arrows), ×200. *c*) In myofibril disarray zones in cardiomyocytes, gap junctions are located in additional intercalated disks, ×400.

the neighboring cells or with diffuse redistribution of gap junctions over the cardiomyocyte surface. In addition, the appearance of additional intercalated disc in cardiomyocytes was observed in areas with marked cardiomyocyte disarray, where the shape of myocytes was changed from cylindrical to polygonal (Fig. 4, *c*) as it was described for the myocardium of patients with HCM [35]. In contrast to patients without cardiovascular pathology, in patients with HCM, the content of Cx43$^+$ gap junctions on the lateral sides of cardiomyocytes also increased relative to the intercalated discs [31]. This change in cell contact topography in the hypertrophied myocardium can be determined by increased number of intercalated disks per individual cardiomyocyte due to additional lateral contacts and increased total area of gap junctions [13].

Changed location of Cx43$^+$ gap junctions was previously detected in the myocardium of patients with various cardiovascular pathologies: periinfarction LV myocardium of CHD patients [19,36], foci of replacement fibrosis in patients with dilated cardiomyopathy [19], and hypertrophied LV myocardium of patients with aortic stenosis [19]. It is believed that remodeling of gap junctions in cardiomyocytes can also be accompanied by abnormal distribution of other components of the intercalated discs, in particular, desmosomes [35], and can be the cause of arhythmogenesis in pathologically altered myocardium [27]. Apart from changes in the localization of gap junctions under pathological conditions, reduction of their number in LV cardiomyocytes was reported in patients with CHD, aortic valve stenosis [18,26,38], chronic hibernation [15], arrhythmogenic cardiomyopathy [25], and dilated cardiomyopathy accompanied by ventricular arrhythmias [16]. A similar decrease in Cx43 expression in subepimyocardium and subendomyocardium by more than 40% in comparison with the control was also described in LV myocardium of dogs with experimental heart failure [7]. Remodeling of gap junctions was also demonstrated in experimental model of HCM in rabbits with a mutation in the β myosin heavy chain gene (β-MyHC-Q403) leading to abnormal transmural distribution Cx43$^+$ gap junctions, in particular, their increased density in the myocardium in comparison with the control [28]. Another model of familial HCM in rabbits with a mutation in cardiac troponin I gene (cTnI^{146Gly}) is associated with increased expression and phosphorylation of Cx43 [32].

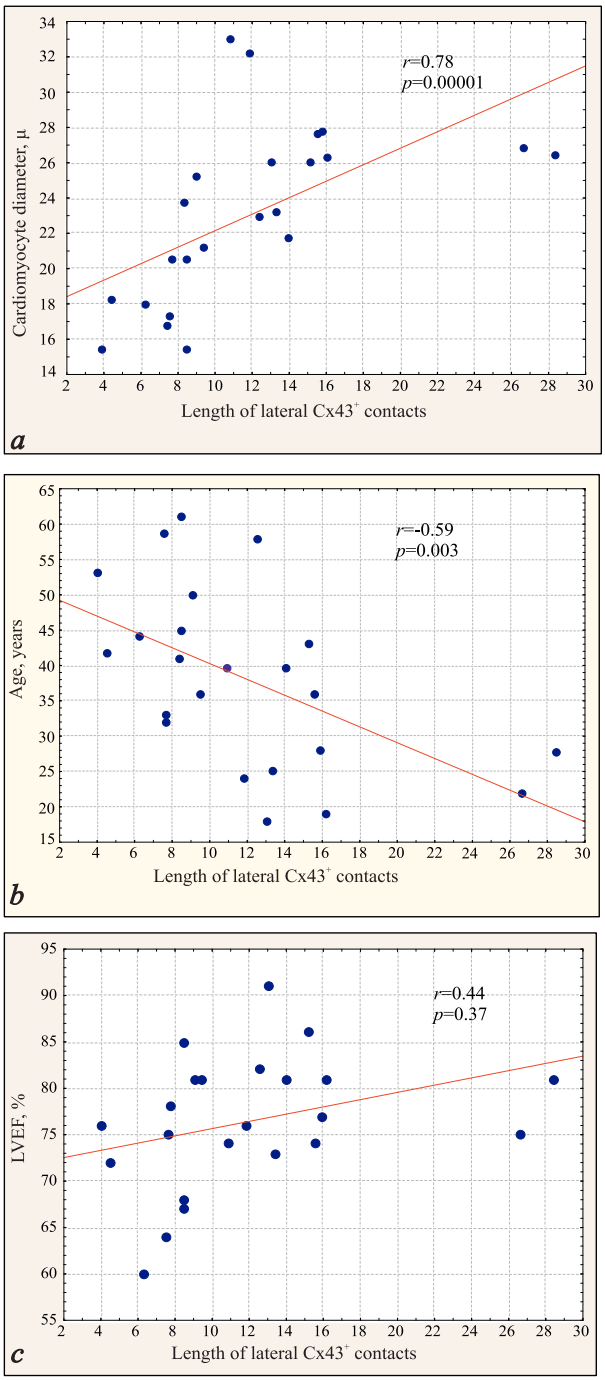

Fig. 5. Clinical and morphological correlations (Spearman's correlation coefficient) of the relative length of Cx43+ gap junctions on the lateral sides of cardiomyocytes (% of doubled length of cardiomyocytes) with the increase in the diameter of IVS cardiomyocyte (*a*), age of patients with HCM (*b*), increase in the LV ejection fraction (LVEF; *c*).

The distribution pattern of Cx43+ gap junctions on the cardiomyocyte surface in this work was also used to assess the myocardium maturity. It is known that in the myocardium of children under 2 years with Fallot tetralogy, gap junctions are distributed over the cardiomyocyte sarcolemma, but in older children, gap junctions are located mainly in the intercalated disks [17,30]. This redistribution of gap junctions is related to cardiomyocyte maturation, differentiation during the ontogeny, and is not associated with the severity of cardiac pathology [30]. Moreover, according to experimental data, age-related decrease in Cx43 expression is regulated at the post-transcription level [10].

According to our findings, the relative length of lateral Cx43+ gap junctions correlated with the increase in cardiomyocyte diameter (r=0.78; p=0.00001) and the loss of myofibrils (r=0.48; p=0.037). Migration of gap junctions on the lateral sides of cardiomyocytes was typical of young patients with HCM (age: r=-0.59; p=0.003), patients with the most severe clinical status, increased ejection fraction (r=0.44; p=0.37), reduced end-diastolic (r=-0.56; p=0.007) and end-systolic size (r=-0.50; p=0.018) (Fig. 1, *d*; Fig. 5). This correlation with the disease severity was previously described in the myocardium of patients with aortic stenosis; in these patients, remodeling of Cx43+ gap junctions was primarily observed during transition from compensated hypertrophy to decompensated status [18,38]. Localization of contacts underwent changes as a part of general remodeling with partial dedifferentiation of cardiomyocytes during which the cells under conditions of energy deficit acquired morphological features typical of immature cardiomyocytes.

There are conflicting reports on the correlation of the degree of myocardial hypertrophy with expression and distribution of Cx43. For instance, comparative study of hypertrophied LV myocardium in patients with aortic stenosis and mitral valve insufficiency revealed no differences in the expression and localization of Cx43 [42]. In contrast, in rats with experimentally induced pulmonary hypertension and hypertrophy of the right ventricle, redistribution of Cx43+ gap junctions on the lateral sides of cardiomyocytes with the corresponding decrease in their number in the intercalated discs were found [33,41]. In hamsters with cardiomyopathy (UM-X7.1) and compensatory LV hypertrophy, reduced expression of Cx43 (mRNA and protein) in the LV myocardium was accompanied by a decrease in conduction velocity [34].

Ischemia and hypoxia of the myocardium also can induce remodeling of gap junctions in cardiomyocytes. In culture of live neonatal rat ventricular cardiomyocytes, migration of Cx43+ gap junctions from the border zones was observed under hypoxic conditions [11]. Similarly, ischemia induced a sharp decrease in the total content of Cx43 in culture of HL-1 mouse atrial myocytes with up to 80% reduction of the population of Cx43+ gap junctions [21].

In turn, our data on proliferative activity of small cardiomyocyte precursors revealed a correlation with morphological parameters of the IVS myocardium

characterizing not only the severity of hypertrophy, but also the degree of cardiomyocyte remodeling and partial dedifferentiation. For instance, a correlation was found between the content of small low early differentiating Ki-67$^+$/sarc α-actin$^+$ cells and an increase in the diameter (r=0.50; p=0.002) and length (r=0.46; p=0.04) of IVS cardiomyocytes and redistribution of Cx43$^+$ gap junctions on the lateral sides of cardiomyocytes (r=0.53; p=0.008) (Fig. 1, *d*). A similar relationship between proliferative activity and the degree of myocardial differentiation was previously demonstrated [37]: the content of resident stem cells and mature differentiated proliferating Ki-67$^+$/cardiac troponin I$^+$ cardiomyocytes increased in experimentally induced hibernation manifested at the morphological level by partial loss of myofibrils.

Thus, critical hypertrophy of IVS myocardium in patients with HCM is determined by not only cardiomyocyte enlargement, but also reactivation of proliferative activity of mature and low-differentiated cardiomyocytes. It seems that proliferative activity of IVS cardiomyocytes in pathologically altered myocardium in HCM is related to structural remodeling of cardiomyocytes (Fig. 1, *d*) characterized by hypertrophy with partial dedifferentiation; morphological manifestations of these processes (partial loss of myofibrils in cardiomyocytes, ultrastructural signs of low differentiated cardiomyocytes, and migration of Cx43$^+$ gap junctions) were observed in our study. Similar cardiomyocyte remodeling is typical of young patients with the most severe (by ultrasound examination data) LV obstruction.

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