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Ultrastructural Criteria of Cardiomyocyte Regeneratory and Plastic Insufficiency in Anthracycline Cardiomyopathy

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We analyzed the dynamics of ultrastructural changes in cardiomyocytes in experimental chronic anthracycline cardiomyopathy. Doxorubicin-induced changes in cardiomyocytes were characterized by a specific combination of ultrastructural changes, which can be regarded as markers of the development of regeneratory and plastic insufficiency. These markers include a triad of changes: deformation of the nuclei with reorganization of the nucleolar system; diffuse and small focal lysis of myofibrils (mainly fine filaments); dilatation of agranular sarcoplasmic reticulum and the intermembrane perinuclear space connected to it. The terminal stages of these disorders are degeneration of some cardiomyocytes, their apoptotic death, and resorption by mononuclear cells (processes representing successive stages in the development of regeneratory and plastic insufficiency of the myocardium).

Key Words: *dilated cardiomyopathy; cardiomyocyte regeneratory plastic insufficiency; ultrastructure; doxorubicin*

Chronic anthracycline cardiomyopathy developing in experimental animals treated with anthracycline antibiotics (doxorubicin (DR) and rubomycin) in sublethal doses is a model of dilated cardiomyopathy of toxic genesis [2,5]. Experimental study of structural mechanisms of dilated cardiomyopathy (at the tissue, cellular, and intracellular levels) showed that heart remodeling by the dilatation variant is caused by the development of regeneratory and plastic insufficiency of cardiomyocytes (CMC), followed by apoptosis and appreciable loss of these cells [1,4]. Diffuse CMC apoptosis can cause a 10-30% decrease in their total count, which serves as the triggering mechanism for dilatation remodeling [2,6,7,12]. Therefore, detection of the main ultrastructural markers of regeneratory and plastic insufficiency of CMC is important for evaluation of the severity of myocardial damage and prediction of heart remodeling.

Here we studied intracellular reorganization of CMC and distinguished the main ultrastructural criteria of regeneratory and plastic insufficiency of these cells during the development of anthracycline cardiomyopathy.

MATERIALS AND METHODS

Anthracycline cardiomyopathy was induced by a single intraperitoneal injection of 10 mg/kg DR hydrochloride (Ferein) to male Wistar rats (n=25). The rats were decapitated 1, 3, 5, 7, 14, 21, and 30 days postinjection. The control group consisted of 10 rats injected with saline intraperitoneally in the appropriate volume simultaneously with experimental animals.

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Specimens of the left-ventricular myocardial tissue were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and then treated routinely [4]. Ultrathin sections were made on an LKB III ultratome, contrasted with uranyl acetate and lead citrate, and examined under a JEM 1010 electron microscope.

RESULTS

The earliest and most demonstrative ultrastructural changes in CMC after injection of anthracycline antibiotics were observed in the nuclear and myofibrillar compartments and agranular sarcoplasmic reticulum (ASR). One day after DR injection and throughout the entire experiment dispersed nucleoli were observed in many CMC nuclei (Fig. 1, *a*); segregation of the fibrillar and granular components of the nucleolonema was noted. In some CMC the nuclei were subsided into the subsarcolemmal zone (Fig. 1, *a*). The nuclear membrane in the majority of nuclei formed appreciable invagination. Five-seven days after DR injection CMC with annular nuclei were seen more and more often (Fig. 1, *b*), which attested to deep suppression of rRNA biosynthesis.

Vacuole-like dilatation of the perinuclear intermembrane space and cisterns and vesicles of the granular reticulum and ASR were seen in CMC with dispersed nucleoli (Fig. 1, *c*). As a result of these changes, the relationship between sarcoplasmic reticulum and perinuclear intermembrane space was clearly seen. Dilated ASR cisterns almost always contained floccular substance.

Doxorubicin-induced ultrastructural reorganization of the nuclear compartment (changed shape, widening of the perinuclear intermembrane space) and ASR (appreciable dilatation of cisterns and vesicles leading to sarcoplasma vacuolation in many cases) could be caused by impairment of the cytoskeleton protein structure and by increased fluidity of CMC membranes [8,10]. These changes in the nuclei accompany disordered biosynthesis of desmin, a cytoskeleton protein belonging to intermediate filaments and participating in the formation of nucleus carcass and involved in signal transduction from the cytoplasm into nuclei [11].

Pronounced changes in the nucleoli (fragmentation and annular shape) correlated with progressing lysis of myofilaments, reduction of organelles, and intensification of autophagocytosis.

Lysis of myofibrils (morphological substratum of contractile insufficiency) appeared within the first 24 h after injection of anthracycline antibiotics. Small focal and diffuse lysis of myofibrillar bundles was seen in the majority of CMC (Fig. 1, *d*) and involved first of all I-bans, containing fine (actin) filaments. Myofibrils became less compact, empty spaces appeared in many sarcomeres, in some cases total lysis of myofilaments within a sarcomere was observed. The most pronounced destruction was observed in sarcomeres of intercalated disks (Fig. 2, *a*) and in the perinuclear zone.

The ultrastructure of mitochondria was little changed. But the appearance of myelin-like structures at sites of mitochondrial accumulation indicates degradation of these organelles (Fig. 2, *b*). Myelin-like structures (bodies) were observed also in the subsarcolemmal zones and near intercalated disks, where they were released into the extracellular space.

Sarcoplasmic matrix in the majority of CMC was appreciably lyzed. Solitary α-glycogen granules, elements of the sarcoplasmic reticulum, and T tubules were well discernible in electron-transparent intermyofibrillar spaces (Fig. 2, *c*). Numerous small lipid droplets and osmiophilic particles located between the mitochondria in the myofibrillar zone were seen in the sarcoplasma of some CMC.

Numerous osmiophilic myelin-like structures appeared in CMC with pronounced destruction of myofibrillar bundles, mitochondria, and partial degradation of the sarcoplasma. Their appearance reflected intensification of autophagocytosis processes in the CMC.

Autophagia of organelles and sarcoplasm was paralleled by their degeneration in the majority of CMC. However, in some cells the appearance of autophagosomes was paralleled by the formation of conglomerations of myofilaments and other organelles, which could be characterized as apoptotic corpuscles. The nuclei of these CMC were condensed and contained mainly heterochromatin; mitochondria often contained electron-dense inclusions. These changes corresponded to ultrastructural signs of apoptosis. Despite accumulation of ribosomes between myofilaments in some CMC with these signs, these cells were destroyed and resorbed by macrophages (Fig. 2, *d*).

Phagosomes with residual filamentous structures and myelin-like bodies were seen in macrophage cytoplasm. It is noteworthy that macrophage cytoplasm often contained numerous lipid inclusions. Active fibroblast forms contained well-developed granular endoplasmic reticulum usually filled with floccular substance with numerous polymorphic mitochondria, polysomes, and well-developed Golgi complex.

Twenty-one days after injection of DR in the sublethal dose the shape of the nuclei was restored in the majority of CMC; the nucleoli also had typical structure. Hypertrophic nucleoli with loop-shaped structure were seen in some CMC with normal ultrastructure. However, dispersed and segregated nucleoli were present in the nuclei of some CMC. Polysomes were seen at sites of myofilament lysis. However, despite re-

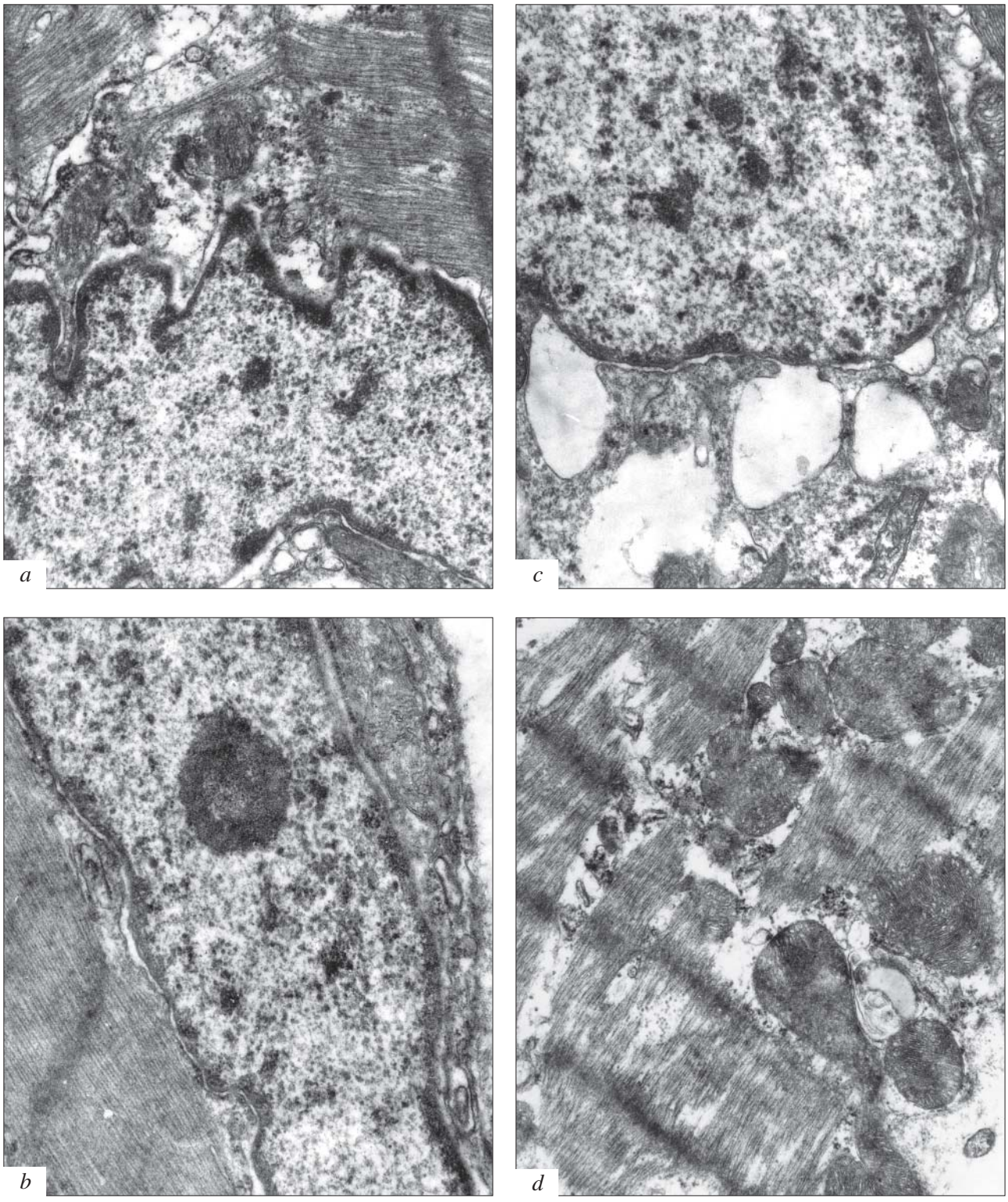

Fig. 1. Ultrastructure of cardiomyocytes during the development of regeneratory and plastic insufficiency induced by anthracycline in Wistar rats. *a*) dispersed nucleolus, invagination of nuclear membrane, and extension of perinuclear intermembrane space, ×10,000; *b*) annular segregation of nucleolar fibrillar and granular components, shifting of nucleus into subsarcolemmal zone, ×15,000; *c*) vacuole-like extension of perinuclear intermembrane space and cisterns of agranular sarcoplasmic reticulum, ×15,000; *d*) diffuse and small focal lysis of myofibrillar bundles, ×10,000.

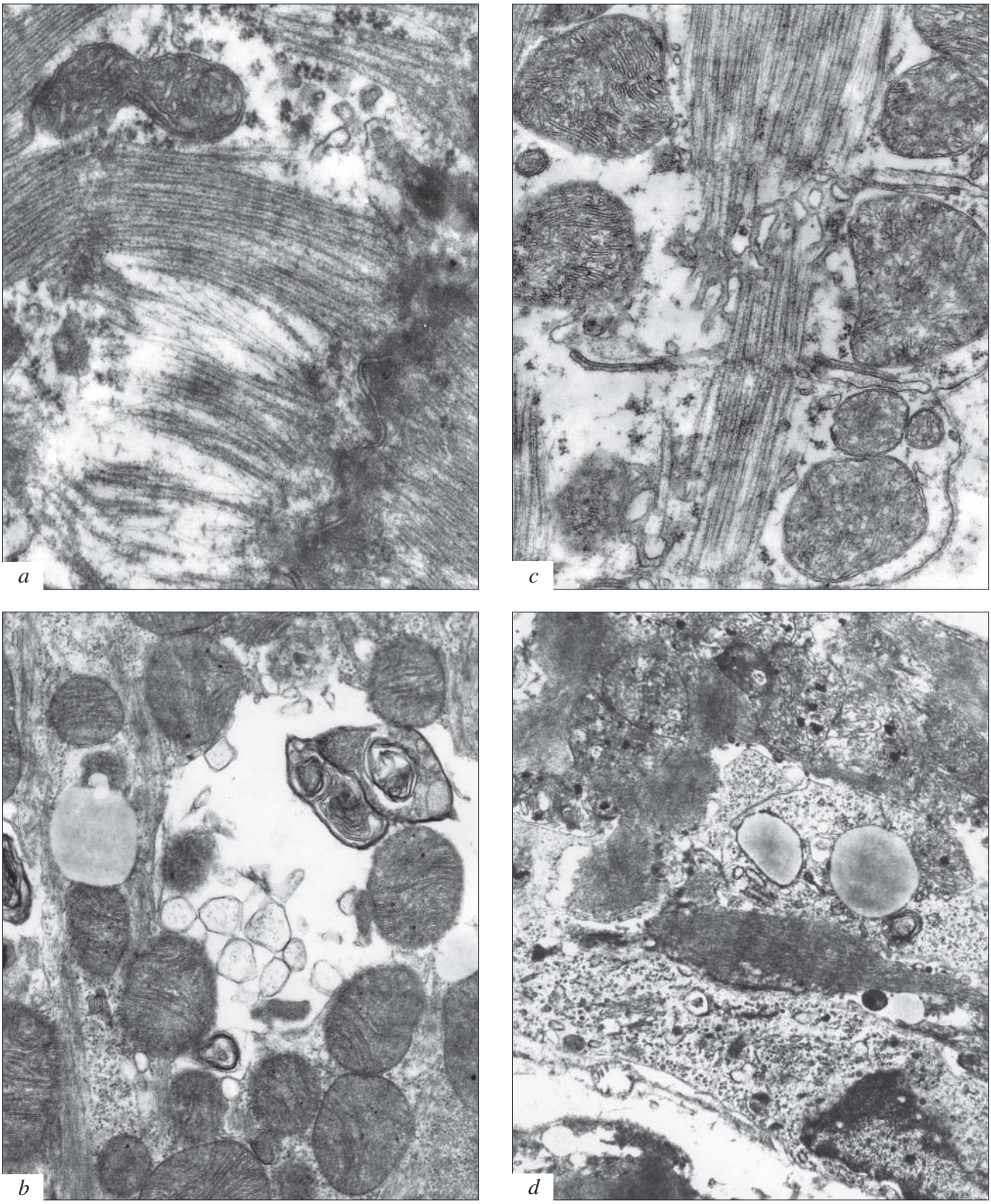

Fig. 2. Ultrastructural manifestations of anthracycline-induced regeneratory and plastic insufficiency in Wistar rat myocardium. *a*) pronounced lysis of myofibrils near intercalated disk, appearance of polysomes, ×20,000; *b*) formation of osmiophilic myelin-like structures in sites of mitochondrial accumulation, ×10,000; *c*) pronounced lysis of myofibrils and sarcoplasmic matrix visualization of agranular sarcoplasmic reticulum, ×15,000; *d*) resorption of apoptotic cardiomyocyte fragments by macrophages, ×5000.

storation of CMC architectonics, the above-described lytic and destructive changes were still observed in some cells: pronounced invagination of the nuclear membrane, diffuse and small focal lysis of myofilaments, extension of perinuclear intermembrane space and ASR.

Thirty days after DR injection the architectonics of most CMC corresponded to normal, but the above-noted ultrastructural changes were still present in some CMC. Autophagy was less pronounced in comparison with earlier periods. Numerous atrophic CMC appeared; hypertrophic CMC were also seen.

The type of ultrastructural changes in the CMC in anthracycline cardiomyopathy reflects some common regularities in the development of their regeneratory plastic insufficiency, which were also observed under the effect of exogenous cholesterol, alimentary deprivation, extreme ecological exposures, and other factors inhibiting biosynthetic processes in cells [3,4].

Summarizing these ultrastructural findings, we distinguish the main causes of contractile disorders in CMC resulting from reduction of intracellular regeneration processes: reorganization of the nuclear compartment paralleled by changes in the shape and size of the nuclei and their translocation into the subsarcolemmal zone; impairment of the architectonics and disorganization of myofibrils, thinning and reduction of myofibrillar bundles; destruction of intercalated disks leading to disorders in transmission of contraction force and dissociation of CMC.

Cardiomyocyte disorders induced by DR and often causing their regeneratory and plastic insufficiency are characterized by marked vacuolation of ASR. Quantitative evaluation of CMC with these changes together with the data on functional activity of the myocardium can also be used as markers of the severity of damage [13].

These ultrastructural changes in CMC are caused in most cases by defects in the cytoskeleton proteins and sarcomeres [9], which result from mutations of the corresponding genes or under the effects of epigenetic factors disordering the biosynthesis processes. CMC with pronounced abnormalities in the nuclear and myofibrillar compartments, ASR, and intercalated disks die through apoptosis, which, in turn, can be the key factor of remodeling by the dilatation variant. An important aspect in structural reorganization of the myocardium in chronic regeneratory and plastic insufficiency is greater phenotypical heterogeneity of CMC and appearance of numerous atrophic and hypertrophic cells.

REFERENCES

1. E. L. Lushnikova, M. G. Klinnikova, O. P. Molodykh, and L. M. Nepomnyashchikh, *Byull. Eksp. Biol. Med.*, **138**, No. 12, 684-689 (2004).
2. E. L. Lushnikova, L. M. Nepomnyashchikh, and V. D. Rozenberg, *Morphological and Molecular Genetic Bases of Dilatational Cardiomyopathy* [in Russian], Moscow (2004).
3. L. M. Nepomnyashchikh, *Regeneratory Plastic Insufficiency of Cardiomyocytes in Impairment of Protein Synthesis* [in Russian], Moscow (1998).
4. L. M. Nepomnyashchikh, E. L. Lushnikova, and D. E. Semenov, *Regeneratory Plastic Insufficiency of the Heart: Morphological Bases and Molecular Mechanisms* [in Russian], Moscow (2003).
5. V. D. Rozenberg and L. M. Nepomnyashchikh, *Dilatational Cardiomyopathy: Common Pathology and Pathomorphology* [in Russian], Moscow (2004).
6. P. Anversa, G. Olivetti, L. G. Meggs, *et al.*, *Circulation*, **87**, 22-26 (1993).
7. P. Anversa, X. Zhang, P. Li, and J. M. Capasso, *J. Clin. Invest.*, **89**, 618-629 (1992).
8. E. L. De Beer, A. E. Bottone, and E. E. Voest, *Eur. J. Pharmacol.*, **415**, 1-11 (2001).
9. D. Fatkin and R. M. Graham, *Physiol. Rev.*, **82**, 945-980 (2002).
10. H. Ito, S. C. Miller, M. E. Billingham, *et al.*, *Proc. Natl. Acad. Sci. USA*, **87**, 4275-4279 (1990).
11. D. J. Milner, G. Weitzer, D. Tran, *et al.*, *J. Cell. Biol.*, **134**, 1255-1270 (1996).
12. B. Nadal-Ginard, J. Kajstura, P. Anversa, and A. J. Leri, *Clin. Invest.*, **111**, 1457-1459 (2003).
13. S. Zhou, A. Starkov, M. K. Froberg, *et al.*, *Cancer Res.*, **61**, 771-777 (2001).