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APOPTOSIS IN SKELETAL MUSCLE

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1. ABSTRACT

Apoptosis is a physiological, conserved program of cellular suicide, characterized by nuclear condensation, DNA-fragmentation and release of mitochondrial cytochrom c into the cytoplasm. The apoptotic program is executed by a cascade of highly specific caspases. The occurrence and significance of apoptosis in multinucleated, differentiated cells, like skeletal muscle cells is discussed controversy in the current literature. In this review we will summarize the knowledge concerning the occurrence of apotosis in certain diseases and the regulation of apoptosis in skeletal muscle.

2. INTRODUCTION

Death within a living tissue may occur by either necrosis or apoptosis. These two processes have fundamentally different morphological appearances, follow different regulatory mechanisms and are of different biological significance (see Table 1). The term apoptosis first appeared in the biomedical literature in 1972, to delineate a structurally-distinctive mode of cell death responsible for cell loss within living tissue (1). The stereotypical morphological features of apoptosis include cell shrinkage, membrane blebbing, breaking up the cells into a number of membrane bound fragments (apoptotic bodies), cytoplasmic condensation, condensation of the nuclear chromatin, and endonuclease-catalyzed degradation of DNA (1). A particular feature of apoptosis is that the plasma membrane of the cell remains intact until very late in the process (1). Changes in several cell surface molecules also ensure that in tissues apoptotic cells are recognized and phagocytosed by their neighbors or macrophages. This phenomenon prevents the activation of inflammatory processes, which may lead to secondary tissue damage (1;2). Furthermore, the apoptotic cell death is a coordinated and an active process requiring ATP as energy source (3;4). Since it has been reported that the loss of mitochondrial membrane potential, which halts mitochondrial ATP production, is an early step in apoptosis (5;6), intracellular ATP required for the rest of the apoptotic pathway must be provided by glycolysis. In contrast to apoptosis disruption of the plasma membrane occurs early in necrosis with consequent inflammation leading to secondary tissue damage.

A number of signaling pathways leading to apoptosis have recently been defined. A common end point of all pathways is the activation of a series of cysteine proteases, also known as caspases (7). The activation of the caspases results in the activation of proteins e.g. CAD

Table 1. Comparison of specific features of apoptosis and necrosis

FEATURES	NECROSIS	APOPTOSIS
Stimuli	Toxins, servere Hypoxia, conditions of ATP depletion	Physiologic and pathological conditions without ATP depletion
Energy requirement	None	ATP-dependent
Histology	Cellular swelling, disruption of organells	Chromatin condensation, apoptotic bodies, death of single isolated cells
DNA breakdown pattern	Randomly sized fragments	Ladder of fragments in internucleosomal multiplies of 185 bp
Plasma membrane	Lysed	intact, blebbed, with molecular alterations (phosphatidylserin externalisation)
Phagocytosis of dead cells	by immigrated phagocytes	by neighboring cells
Tissue reaction	Inflammation	no inflammation

(caspase activated DNase) finally leading to cell death.

Apoptosis is known to play an important physiological role during embryonic development and in the control of the cell number in proliferative tissues. In addition it has been described in mature brain (8;9), heart (10-14), and thymus (15) in response to specific insults.

While considerable knowledge has been accumulated on the regulation of apoptosis in immune-competent cells the actual process of programmed cell death (PCD) is poorly understood in solid, differentiated tissues such as the skeletal muscle (16). Defining the relative importance of nuclear apoptosis in muscle fiber death is substantially complicated by the presence of hundreds of myonuclei in each muscle fiber. Some nuclei in a single cell can be targeted for death while others are unaffected (17).

The purpose of this review is to summarize the knowledge in the current literature concerning the occurrence of apoptosis in mature skeletal muscle and the molecular regulation of this process.

3. METHODS FOR THE DETECTION OF APOPTOSIS

The detection of apoptosis is based on two hallmarks of the process: typical morphological changes (1) and internucleosomal DNA fragmentation (18) (see Table 2).

3.1. Morphological Methods

Microscopic methods like electron microscopy, confocal microscopy (19) or light microscopy are most commonly applied to detect morphological alterations of the cell nucleus. Using electron microscopy apoptosis was detected in myocardial tissue in the case of a degenerating conduction system (19), in experimental heart failure (20), and in the human hibernating myocardium (21). A major limitation of electron microscopy is that the detection of single apoptotic cell nuclei is very difficult and can be compared to finding a needle in a hay stack. Moreover, cardiomyocytes and skeletal muscle myocytes do not exhibit the classical nuclear morphology associated with apoptosis, despite observed DNA fragmentation (22;23). Therefore, most of the evidence on the occurrence and quantity of apoptosis was obtained by methods using light microscopy. In living cells, it is possible to detect the externalization of phosphatidylserine in apoptotic cells by Annexin 5 labeling. The specific staining can be further evaluated by either conventional light microscopy or by FACS (flouresence activated cell sorter)-analysis. In some tissues, apoptotic cells can be identified by routine histological staining or using specific nuclear stains, such as propidium iodine or Hoechst stain, but usually special techniques are applied to detect biochemical or molecular markers of apoptosis.

3.2 Biochemical and Molecular Methods

One of the most applied method to prove apoptosis in tissue section is the TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labeling) reaction (24). This method labels the free 3´-ends of DNA by terminal transferase (TNT), and the label is then visualized by immunohistochemical techniques. Nevertheless, the TUNEL reaction seems to be prone to false positive or negative findings and several improvements of the methods have been proposed (25;26). The staining is very dependent on fixation time of the tissue samples (27), proteolytic pretreatment of the section (28;29) and the concentration of the nucleotides and terminal transferase used for labeling (29). A recent analysis demonstrated that most of TUNEL-positive myocytes of patients with dilated cardiomyopathy are not apoptotic but rather living cells with increasing activity of DNA-repair (30). Therefore, the TUNEL-method, as used by the majority of investigators, has to be regarded with caution concerning its specificity.

Apart from the TUNEL reaction several other immunological methods have been developed to detect and quantify apoptosis related proteins, such as Bcl-2, protooncogene Bax, Bcl-Xl, the caspase induced cleavage of PARP (poly-ADP-ribose polymerase), mitochondrial release of cytochrome c, or the cleavage of procaspase-3 (31-34). The disadvantage of all these determinations is that they have to be considered only as indirect evidences of apoptosis.

Another frequently used assay based on the second hallmark of apoptosis is the separation of cellular DNA on agarose gels. This technique takes advantage of the fact, that during the apoptotic process the DNA is cleaved at sites located between nucleosomal units, thereby generating DNA mono- and oligonucleosomal fragments (180bp multimers), which may be visualized on agarose gels (DNA-laddering

Table 2. Comparison of different methods to detect apoptosis

Method	Parameter analyzed	Sensitivity	Specifity	Adavantages	Disadvantage
TUNEL	DNA stand breaks	high	low	- usefull for fixed or frozen tissue sections - identification of single nuclei and assignment of apoptotic nuclei to cell type is possible	labor intensive, only a few tests may be performed simultaneously
DNA-ladder	DNA fragmentation	low	high	a clear hallmark of apoptosis	- no assignment of the apoptotic nuclei to a specific cell type - no quantitative measurement
Electron microscopy	Nuclei morphology	high	moderate	- a clear hallmark of apoptosis - assignment of apoptotic nuclei to cell type is possible	- very labor intensive - difficult to detect few cells in a large cell population - not all apoptotic cells show morphological changes
Protease activity assay	Activation of caspase-3	low	moderate	- Marker for very early stage of apoptosis	a high number of apoptotic cells are required for a positive result

assay). The disadvantage of this assay is that it is difficult to quantify the apoptotic process, and that enough tissue material has to be available to isolate the DNA. Furthermore, cleavage of chromatin to nucleosomal fragments does not occur in all cell types and can be inhibited without blocking the other features of apoptosis (35;36).

In conclusion, as long as no gold standard for the specific detection of apoptosis is available, it seems that it is very important not to rely on a single method to detect or even quantify apoptosis.

4. OCCURRENCE OF APOPTOSIS IN MATURE SKELETAL MYOCYTES

Muscle cell death is observed in various physiological and pathophysiological circumstances. For example, during differentiation the tail muscle of tadpoles is fragmented via apoptosis and large chunks of muscle are phagocytosed (37). The question arises, however, whether apoptosis is also evident in end-differentiated skeletal myocytes. In the following section, we want to summarize the knowledge about the occurrence of apoptosis in differentiated skeletal muscle fibers in different pathological conditions and its physiological relevance.

4.1. Skeletal Myocyte Apoptosis in Duchenne Muscular Dystrophy (DMD)

Duchenne muscular dystrophy, one of the best characterized muscle disease, involves muscle wasting and the premature death of afflicted patients as a consequence of respiratory failure. Although the primary defect in dystrophic muscle is very well characterized (38), the relationship between the absence of the membrane-associated, cytoskeletal protein dystrophin and cell death is not completely solved. Several studies have analyzed the occurrence of apoptosis in skeletal muscle samples obtained from patients with DMD or from the mdx-mouse, an animal model of the disease. Many characteristics of cell death in dystrophin deficient muscles resembles those of necrosis, such as pathological elevation of intracellular calcium, increase in the sarcoplasmic volume, aberrant morphology of the mitochondria, and inflammation (39-43). At that time the working model of myocyte death via necrosis was not able to explain the relative late clinical onset of muscle pathology, at about 3 years of age in DMD-patients and 3-4 weeks in the mdx-mice. There had to be a trigger for cell death between the absence of dystrophin and the death of muscle cells. In 1995 two papers demonstrated for the first time, that in the skeletal muscle of the mdx-mouse apoptosis precedes necrosis, and that the apoptotic events continued into the stage of dystrophic pathology, that is currently viewed as necrosis (17;44). This observation could be confirmed by several other groups in the mdx-mouse (45-48). In skeletal muscle biopsies obtained from DMD patients the results concerning the presence of apoptotic myonuclei is still controversial. In recent studies it has been demonstrated that not only infiltrating macrophages but also muscle fibers were stained apoptosis positive using TUNEL methodology (47;49). These results, however, were not confirmed in studies using DNA-laddering for detection of apoptosis in myogenic cells (50;51). It is noteworthy, that all the TUNEL-labeling detected was restricted to mononuclear infiltrates. Furthermore, Sandri and coworkers could demonstrate that the apoptotic index in mdx-mice dramatically increased after physical exercise, whereas in the skeletal muscle of control animals only a minor increase of apoptotic cells was detectable (46). This observation would be an explanation for the observed exercise-induced muscle damage and its influence on the progression of the disease.

4.2. Skeletal Myocyte Apoptosis in Atrophy due to Unloading or Motor Neuron Disorders

Reduction of neuromuscular activity and/or unloading, by denervation, hindlimb suspension, or zero gravity during spaceflight, results in rapid and substantial atrophy of skeletal muscle fibers (52). The mechanisms, by which muscle fiber size is reduced includes transcriptional and posttranslational pathways (53;54).

Several studies demonstrated, that muscle atrophy associated with unloading is accompanied by a reduction in mean number of myonuclei per fiber (55-57). Even before the term ”apoptosis” was introduced into science (58), Lee and

Altschel observed a number of myonuclear ultrastructural abnormalities after long-term denervation, including chromatin condensation, nuclear shrinkage, and nuclear fragmentation (59). Investigating the question if the elimination of myonuclei during atrophy either by denervation or unloading is due to apoptosis, Allen and colleagues analyzed rat skeletal muscle samples after hindlimb unweighting by TUNEL- and Hoechst-staining. (60). In their analysis they documented that the mean number of TDT-myonuclei was significantly increased after 3 days of suspension, and that this number remained consistent throughout the 14-day period. However, the biochemical signals regulating myonuclear destruction by apoptosis are not known. An attractive working model, that the regulation of myonuclear number is dependent upon the interactive effects of circulating growth hormones/IGF-1 is supported by the following observations. McCall et al. reported that muscle atrophy induced by unloading is associated with a decrease in the circulating level of bioassayable growth hormone (61). Furthermore, treatment of hindlimb suspended muscles with IGF-1 in conjunction with exercise significantly reduced the frequency of apoptotic myonuclei (60).

4.3.Skeletal Myocyte Apoptosis in Ischemia/Reperfusion

Ischemia, with or without subsequent reperfusion, is a common and clinically important cause of injury in many tissues. Apoptosis has been observed in hypoxic endothelium cells (62), and in cells from different tissues, like the intestine (63), and heart (64;65) following ischemia-reperfusion injury.

Current data about apoptosis in skeletal myocytes after ischemia/reperfusion are still controversial. Hachiya and Kazui reported TUNEL-positive cells and a DNA ladder pattern after reperfusion, concluding that cell death due to ischemia/reperfusion involves not only necrosis but also apoptosis (66). On the other hand, Knight and coworkers reported the presence of apoptotic cells after 30 min of reperfusion of the rat gastrocnemius muscle (67). An analysis of the apoptotic cells clearly demonstrated that only endothelial and smooth muscle cells as well as neutrophils and macrophages were TUNEL-positive, whereas skeletal muscle fibers were free of apoptosis positive nuclei.

In conclusion, it is still unclear if during ischemia/reperfusion skeletal muscle fibers die by necrosis or apoptosis, and if apoptotic cell death is restricted to neutrophils and macrophages infiltrating the damaged muscle.

4.4. Skeletal Myocyte Apoptosis in Chronic Heart Failure (CHF)

Chronic heart failure is not only associated with an increased mortality, but also with exercise intolerance and early fatigue. It has been frequently pointed out, however, that the level of activity tolerated by individual patients could not be predicted by classical parameters of left ventricular performance (68;69)**.** Therefore, considerable attention has been focused on the role of peripheral factors such as regional blood flow and skeletal muscle, as determinants of work capacity. Based on the observation of myocardial apoptosis in dilated cardiomyopathy, the question arises whether apoptosis is also prevalent and relevant in the peripheral skeletal muscle of heart failure patients. Our group and the group of Vescovo investigated the occurrence and significance of apoptosis in skeletal muscles of patients with CHF and in a CHF animal model (70-72).

We analyzed for the first time the presence of apoptotic nuclei in human skeletal muscle biopsy material of CHF patients by TUNEL. TUNEL-positive nuclei were detected in 47% of CHF patients and we could demonstrate by counterstaining with anti-actin that apart from infiltrating immune cells, also myonuclei proved to be apoptosis positive (73). Evaluation of apoptosis positive muscle specimens revealed an apoptotic index of 0.7 ± 0.4%. Furthermore, the occurrence of apoptosis correlated with a reduced exercise capacity (VO_2max), suggesting a functional significance of apoptosis for exercise intolerance of CHF.

In an animal model Vescovo and coworkers demonstrated, that after monocrotaline induced right ventricular heart failure the number of TUNEL positive myonuclei in the fast twitch muscle tibialis anterior increased significantly over time compared to control rats (after 27 days; 0.0025 ± 0.005% vs. 0.031 ± 0.012%). The increase in apoptosis was accompanied by muscle atrophy evident by a drop in fiber cross-sectional area and muscle weight/body weight. Furthermore, in a follow-up report the same group demonstrated that also in slow twitch soleus muscle apoptosis was evident in myonuclei and that the number increased over time (71). Nevertheless, the apoptotic index in the soleus muscle was lower and no signs of atrophy could be observed as compared to tibialis anterior. The validity of the monocrotaline model as animal model for chronic heart failure and apoptosis has to be questioned, because in an in-vitro cell culture assay a low as well as a high concentration of the reactive metabolite monocrotaline pyrrole induced apoptotic cell death in arterial endothelial cells (74). Therefore, the conclusion that the observed apoptotic cell death in skeletal muscle fibers is due to heart failure needs to be confirmed in other animal models of CHF.

Based on the available human and animal studies its seems that in CHF apoptosis occurs in skeletal muscle, and that it may have an influence on muscle atrophy and contractility.

Taken all the results from studies analyzing various diseases, one must conclude that apoptosis is evident also in multinucleated skeletal muscle cells. However, does apoptosis represents only an epiphenomenon without pathophysiological significance (bystander effect) or is it important for muscle dysfunction?

5. INCIDENCE AND SIGNIFICANCE OF APOPTOSIS IN SKELETAL MUSCLE

Presently, the debate about the pathophysiological relevance of myocyte apoptosis in certain diseases deals with quantitative questions. Reported data on the occurrence of skeletal muscle apoptotic nuclei in human or animal disease models range between 0.03 % (70) and 2.1% (47). To enter the

discussion about significance and relevance of apoptosis in skeletal muscle, it is important to solve the question of how long is an apoptotic nucleus detectable and what would be the relevance of such a small loss of nuclei for myocyte integrity and function ?

Concerning the time frame, in which an apoptotic nuclei is detectable, time course studies on dexamethasone-stimulated thymocytes have shown, that, using the TUNEL technique, apoptotic nuclei are detectable for approximately 1 to 3 hours after the onset of apoptosis (24). This is in part due to rapid phagocytosis of dead cells and apoptotic fragments. At the moment it is completely uncertain if it is possible to transfer these results of the *in-vitro* assays to the *in-vivo* situation. Without the knowledge of how fast a nuclei or even a cell is removed in the tissue, it is very difficult to assess the apoptotic indices published in the recent literature.

Is a low apoptotic index in a certain tissue relevant for its function? Based on observations made by Howie and colleagues a low frequency of apoptosis may still represent a major cell turnover (75). They demonstrated that the depletion of CD4 cell in mouse lymph nodes by 50% can be achieved by apoptosis within 48h, but the observed apoptotic rate never rose above 1.3%. Therefore, a small number of apoptotic nuclei at one time detected in skeletal muscle in different diseases may contribute to a substantial loss of muscle mass over a longer time period, resulting in a decreased contractility (47;73).

The discussion on relevance and significance of apoptosis in skeletal muscle is further complicated by the fact that in each muscle fiber more than 100 nuclei are present, and that only a minority of myonuclei inside the myofiber display DNA fragmentation (nuclear death) (60;76). Does the loss of a single or of several nuclei alter fiber morphology or even function? Based on the concept that one nucleus controls a specific territory (nuclear domain), one has to assume that the loss of a single nucleus is associated with the loss of the controlled cytoplasmic territory (77;78). This hypothesis was confirmed by Hikida and coworkers (57). They analyzed the myonuclear population in the soleus muscle of rats that had undergone atrophy due to 10 days spaceflight, and could demonstrate that the number of nuclei was reduced proportionally to the loss of fiber size.

In mononucleated cells, the complete apoptotic cell is removed by phagocytosis, whereas in multinucleated cells only the apoptotic nucleus is removed leaving the rest of the cell intact. At the present time it is not completely understood how an individual myonucleus can be eliminated without destruction of all nuclei or the entire myocyte (nuclear death). Nevertheless, studies using heterokaryons of thymocytes and NIH3T3 cells demonstrated, that individual nuclei can indeed undergo disintegration without destroying neighboring nuclei or the entire cell (79).

Taking all these results together, one has to conclude that apoptosis occurs also in differentiated skeletal muscle, but the extent and the significance for physiological alterations still remain to be determined.

6. MOLECULAR MECHANISM FOR THE REGULATION OF THE APOPTOTIC PROCESS

Over the last years a considerable body of knowledge accumulated on the regulation of apoptosis in immunecompetent cells (reviewed in (80;81) (Figure 1). On the other hand in solid tissues, and especially in multinucleated cells like skeletal muscle, the initiation and regulation of apoptosis is still poorly understood. In the following section we summarize the knowledge about different important factors of the apoptotic cascade in skeletal muscle cells.

6.1. Caspases

Apoptosis can be activated endogeneously through a genetically defined program or by exogenous proteins, cytokines and hormones, as well as xenobiotic compounds (e.g. radiation, oxidative stress, and hypoxia). A central component of the signal mechanism leading to apoptotic cell death is the activation of a series of procaspase proteases (82). In terms of skeletal muscle cells, the available data on caspase activity is limited. Yashuhara and coworkers investigated the occurrence of apoptosis after burn injury (83). They could show, that beneath the burned surface, as well as in muscles at sites distant from the burn injury, caspase-1,-3, and -9 were activated and myocyte apoptosis was detectable.

In a different experimental setting skeletal muscle fibers of mice exhibiting a dystrophic skeletal muscle due to a deficiency of the laminin alpha2 protein were analyzed for caspase-3 activation and induction of apoptosis (84). Using a cleavage site directed caspase-3 antibody it could clearly be demonstrated that caspase-3 is activated in the skeletal muscle of these mice as compared to wild-type mice.

Based on these limited studies one may conclude, that the activation of caspase-3 is part of the activation cascade leading to apoptotic cell death, as it is described for several other tissues (85;86)

6.2. Mitochondria and the Release of Apoptotic Regulating Substances

Disturbances of mitochondrial function induced by several different factors e.g. toxins are associated with changes in membrane potential. After the disruption of the mitochondrial membrane potential, the conductance of the mitochondrial permeability transition pore (PT) is increased, and cytochrome c as well as AIF (apoptosis inducing factor) are released into the cytosol. This permeability increase can be prevented by the protooncogene Bcl-2 (87-90), which is located in the mitochondrial outer membrane (87). In the cytosol cytochrome c activates the oligomerization of APAF-1 (91-93), a mammalian homologue of the Caenorhabditis elegans cell death protein ced-4 (94). Once activated APAF-1 binds to and activates procaspase-9. The complex formed between cytochrome c, APAF-1 and procaspase-9 is often designated in the current literature as ”apoptosom” (95).

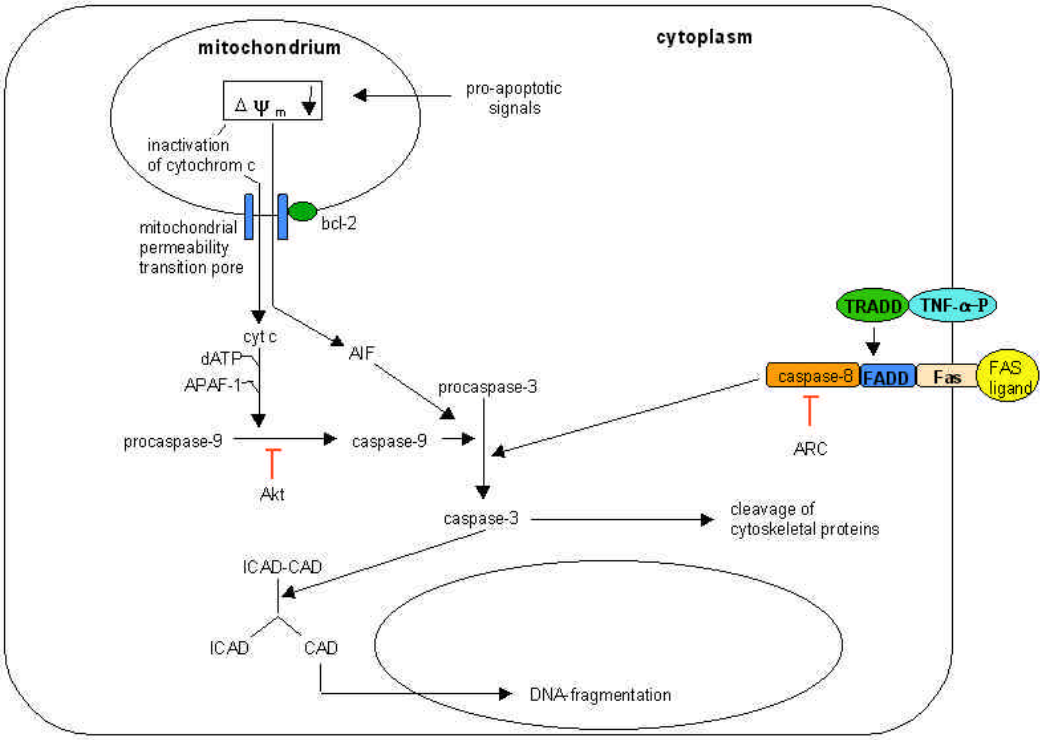

Figure 1. Possible pathways for the regulation of apoptosis are illustrated. Pro-apoptotic signals induce mitochondrial permeability transition resulting in a release of cytochrome c (cyt c) and AIF (apoptosis inducing factor) into the cytoplasma. AIF activates caspase 3, whereas cyt c activates the oligomerization of APAF (apoptosis protease activating factor) leading to the activation of caspase 9 and 3. Activated caspase 3 cleaves the ICAD (inhibitor of CAD) and free CAD (caspase activated Dnase) cleaves DNA. An other possible route for activation of apoptosis is via death receptors like TNF-a or FAS. Once activated these receptors lead to the activation of caspase 8 and caspase 3. The activation of caspase 8 is inhibited by ARC (apoptosis repressor with caspase recruitment domain), whereas AKT (AKT-kinase) is a possible inhibitor of the caspase 9 activation.

Is the mitochondrial pathway involved in the activation of apoptosis in skeletal muscle? Concerning the release of cytochrome c into the cytoplasm, no experimental data on skeletal muscle are available up today. However, for cardiomyocytes a 15 to 20 fold higher concentration of cytochrome c in the cytoplasmic compartment could be demonstrate in 16 explanted hearts, resulting in an activation of caspase 3 (34). Therefore, at least in heart muscle, changes in the mitochondrial permeability may be involved in the execution of the programmed cell death.

6.3. APAF-1

Besides the release of cytochrome c, APAF-1 is an important factor for the activation of procaspase 9 via cytochrome c. A recent report from Burgess and coworkers demonstrated that APAF-1, a central player in the mitochondrial dependent activation of caspase-3, is not detectable in skeletal muscle of healthy volunteers at the protein level and only very weak at the transcriptional level (19;92). However, nothing has been reported so far concerning the expression of APAF-1 in pathophysiological situations where apoptosis is detected in skeletal muscle.

6.4. Bcl-2 family

The release of mitochondrial apoptogenic factors such as cytochrome c and AIF into the cytoplasm is controlled by the Bcl-2 family of apoptosis regulators. Mitochondria purified from Bcl-2 overexpressing cells are protected against the mitochondrial permeability transition induced release of cytochrome c and AIF and the subsequent induction of the apoptotic cell death (88;90;96). On the other side, the overexpression of Bax causes a dissipation of the mitochondrial membrane potential, thereby initiating the mitochondrial controlled apoptotic pathway. Several reports in the literature have analyzed the expression between the pro- and antiapoptotic Bcl-2 family members. They all could demonstrate that in different diseases, where apoptosis could be detected in skeletal myocytes, the expression of Bcl-2 was unchanged or reduced whereas the Bax-expression increased, thereby shifting the balance between pro- and antiapoptotic factors towards the proapoptotic side (46;47;70;73).

6.5. Nitric Oxide

Nitric oxide (NO) represents another possible factor inducing the apoptotic pathway in skeletal muscle. It has been demonstrated that NO has the potential to induce

apoptosis in cell culture systems of mouse thymocytes (97), macrophages (98), rat ventricular myocytes (99) as well as in rat skeletal muscle myoblast (100). Recently, the expression of the inducible isoform of nitric oxide synthase (iNOS) has been demonstrated in skeletal myocytes of patients with chronic heart failure (101;102). The expression of iNOS correlated inversely with the exercise capacity of CHF patients (103). We could recently demonstrate that apoptosis occurred in skeletal muscle of roughly 40% of the patients and that patients exhibiting apoptosis positive skeletal muscle biopsies showed a higher expression of iNOS (73). These results indicate that nitric oxide is a potential stimulus for apoptosis in skeletal muscle of patients with chronic heart failure.

What may be the mechanism by which NO is inducing apoptosis? One possible mechanism would be that NO induces apoptosis via peroxinitrite, a potent proapoptotic reaction product of NO and superoxide (99). An other mitochondrial dependent pathway by which NO may induce apoptosis has been recently discussed by Hortelano and coworkers (104). They showed that NO increases the mitochondrial membrane potential and chemically modifies cytochrome c, thereby altering its structure and facilitating its release from the mitochondria into the cytosol finally leading to caspase-3 activation.

7. SUMMARY AND POTENTIAL IMPLICATIONS

Although evidence has been provided that apoptosis is a feature in skeletal muscle fibers in several disease like chronic heart failure or Duchenne muscular dystrophy, there are still several unsolved issues that need to be addressed.

1. Development/refinement of methods with high sensitivity and specificity for the detection of apoptosis. At the present time no gold standard for the proof of apoptosis is available making it very hard to compare different studies with each other. In many previous studies the TUNEL or DNA laddering methodology was used to detect and even quantitate apoptosis. As documented now by recent studies TUNEL staining and DNA laddering may not suffice to prove apoptosis and other alterations are probably required to confirm apoptosis.

2. Determination of functional significance of small numbers of apoptotic nuclei in a multinucleated skeletal muscle fiber. Almost all published studies of apoptosis in human or in animal models of different diseases are descriptive verifying the presence of apoptotic cells, while the relation to function outcome remains mostly unknown.

3. Analysis of molecular mechanisms involved in the regulation of apoptotic cell death. At the molecular level it still remains uncertain which mechanisms initiate the apoptotic process in skeletal muscle. The definition of the mechanism will also help to define potential targets for future interventions. In the current literature there are studies which only describe the activation of members of the apoptotic pathway e.g. caspases but the exact involvement of these parameters for the initiation of apoptosis is still unknown.

When all these issues have been adequately addressed anti-apoptotic therapeutic strategies may be developed for systemic myopathies in which apoptosis plays a central role.

8. REFERENCES

1. Kerr JFR, A.H. Wyllie & A.R. Currie: Apoptosis:A basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257 (1972)

2. Bursch W, S. Paffe, B. Putz & R. Schulte-Hermann: Determination of the lenght of the histological stages of apoptosis in normal liver and in altered hepatic foci of rats. *Carcinogenesis* 11, 847-853 (1990)

3. Kroemer G, B. Dallaporta & M. Resche-Rigon: The Mitochondrial Death/Life Regulator in Apoptosis and Necrosis. *Annu.Rev.Physiol.* 60, 619-642 (1998)

4. Tsuijmoto Y: Apoptosis and necrosis: Intracellular ATP level as a determinant for cell death modes. *Cell Death and Differ.* 4, 429-434 (1997)

5. Shimizu S, Y. Eguchi, W. Kamiike, S. Waguri, Y. Uchiyama, H. Matsuda & Y. Tsujimoto: Bcl-2 blocks loss of mitochondrial membrane potential while ICE inhibitors act at a different step during inhibition of death induced by respiratory chain inhibitors. *Oncogene* 13, 21-29 (1996)

6. Zamzami N, P. Marchetti, M. Castedo, C. Zanin, J.L. Vayssiere, P.X. Petit & G. Kroemer: Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. *J Exp Med* 181, 1661-1672 (1995)

7. Thornberry NA & Y. Lazebnik: Caspases: Enemies within. *Science* 281, 1312-1316 (1998)

8. Heron A, H. Pollard, F. Dessi, J. Moreau, F. Lasbennes, Y. Ben-Ari & C. Charriaut-Marlangue. Regional variability in DNA fragmentation after global ischemia evidenced by combined histological and gel electrophoresis observation in the rat brain. *J Neurochem* 61, 1973-1976 (1993)

9. Linnik MD, R.H. Zobrist & M.D. Hatfield: Evidence supporting a role for programmed cell death in focal cerebral ischemia in rats. *Stroke* 24, 2002-2009 (1993)

10. Gottlieb RA, K.O. Burleson, R.A. Kloner, B. Babior & R.L. Engler: Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J.Clin.Invest.* 94, 1621-1628 (1994)

11. Buerke M, T. Murohara, C. Skurk, C. Nuss, K. Tomaselli & A.M. Lefer: Cardioprotective effect of insulin-like growth factor 1 in myocardial ischemia followed reperfusion. *Proc Nat Acad Sci* 92, 8031-8035 (1995)

12. Mallat Z, A. Tedgui, F. Fontaliran, R. Frank, M. Durigon & G. Fontaine: Evidence for apoptosis in arrhythmogenic right ventricular dysplasia. *N Engl J Med* 335, 1190-1196 (1996)

13. Narula J, N. Haider, R. Virmani, T.G. DoSalvo, F.D. Kolodgie, R.J. Hajjar, U. Schmidt, M.J. Semigran, G.W. Dec & B.A. Khaw: Apoptosis in myocytes in end-stage heart failure. *N Engl J Med.* 335, 1182-1189 (1996)

14. Kuribayashi-Ohta K, S. Tamatsukuri, M. Hikata, C. Miyamoto & Y. Furuichi: Application of oilgo(dT)30-latex for rapid purification of poly(A)+ mRNA and for hybrid substraction with the *in situ* reverse transcribed cDNA. *Biochim Biophys Acta* 1156, 204-212 (1993)

15. Ayala A, C.D. Herdon, C.M. DeMaso, C.A. Ayala & I.H. Chaundry: The induction of accelerated thymic programmed cell death during polymicrobal sepsis, Control by corticosteroids but not tumor necrosis faktor. *Shock* 3, 259-267 (1995)

16. Burgess DH, M. Svensson, T. Dandrea, K. Grönlund, F. Hammarquist, S. Orrenius & I.A. Cotgreave: Human skeletal muscle cytosols c-dependent activation of type-2 caspases and lack APAF-1. *Cell Death and Differ* 6, 256-261 (1999)

17. Tidball JG, D.E. Albrecht, B.E. Lokensgard & M.J. Spencer: Apoptosis precedes necrosis of dystrophin-deficient muscle. *J Cell Science* 108, 2197-2204 (1995)

18. Wyllie AH: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284, 555-556 (1980)

19. James TN, F. Terasaki, E.R. Pavlovich & A.M. Vikhert: Apoptosis and pleomorphic micomitochondriosis in the sinusnodes surgically excised from five patients with the long QT syndrome. *J Lab Clin Med* 122, 309-323 (1993)

20. Saraste A, K. Pulkki, M. Kallajoki, K. Henriksen, M. Pavinen & L.M. Viopio-Pulkki: Apoptosis in human acute myocardial infarction. *Circulation* 95, 320-323 (1997)

21. Elsasser A, M. Schlepper, W.P. Klovekorn, W.J. Cai, R. Zimmermann, K.D. Muller, R. Strasser, S. Kostin, C. Gagel, B. Munkel, W. Schaper & J. Schaper: Hibernating myocardium, an incomplete adaptation to ischemia. *Circulation* 96, 2920-2931 (1997)

22. Ohno M, G. Takemura, A. Ohno, J. Misao, Y. Hayakawa, S. Minatoguchi, T. Fujiwara, & H. Fujiwara. Apoptotic myocytes in infarct area in rabbit hearts may be oncotic myocytes with DNA fragmentation. Analysis by immunogold electron microscopy combined with *in situ* nick end-labeling. *Circulation* 98, 1422-1430 (1998)

23. Gottlieb RA, K.O. Burleson, R.A. Kloner & R.L. Engler: Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 94, 1621-1628 (1994)

24. Gavrieli Y, Y. Sherman & S.A. Ben-Sasson: Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. *J Cell Biol* 119, 493-501 (1992)

25. Negosecu A, P. Lorimier, F. Labat-Moleur, C. Drouet, C. Robert, C. Guillermet, C. Brambilla & E. Brambilla: *In situ* apoptotic cell death labeling by the TUNEL method, improvement and evaluation on cell preparations. *J Histochem Cytochem* 44, 959-968 (1996)

26. Labat-Moleur F, C. Guillermet, P. Lorimier, C. Robert, S. Lantuejoul, E. Brambilla & A. Negoescu: TUNEL apoptotic cell detection in tissue sections, critical evaluation and improvement critical evaluation and improvement. *J Histochem Cytochem* 46, 327-334 (1998)

27. Davison FD, M. Groves & F. Scaravilli: The effects of formalin fixation on the detection of apoptosis in human brain by *in situ* end labelling of DNA. *Histochem J* 27, 983-988 (1995)

28. Negoescu A, P. Lorimier, F. Labat-Moleur, C. Drouet, C. Robert, C. Guillermet, C. Brambilla & E. Brambilla: *In situ* apoptotic cell labeling by the TUNEL method, improvement and evaluation on cell preparations. *J Histochem Cytochem* 44, 959-968 (1996)

29. Migheli A, P. Cavalla, S. Marino & D. Schiffer: A study of apoptosis in normal and pathologic nervous tissue after *in situ* end-labeling of DNA strand breaks. *J Neuropathol Exp Neurol* 53, 606-616 (1994)

30. Kanoh M, G. Takemura, J. Misao, Y. Hayakawa, T. Aoyama, K. Nishigaki, T. Noda, T. Fujiwara, K. Fukuda, S. Minatoguchi & H. Fujiwara: Significance of myocytes with positive DNA *in situ* nick end-labeling (TUNEL) in hearts with dilated cardiomyopathy, not apoptosis but DNA repair. *Circulation* 99, 2757-2764 (1999)

31. Saraste A: Morphologic criteria and detection of apoptosis. *Herz* 24, 189-195 (1999)

32. Duriez PJ & G.M. Shah: Cleavage of poly(ADP-ribose)polymerase, a sensitive parameter to study cell death. *Biochem Cell Biol* 75, 337-349 (1997)

33. Sgonc R & J. Gruber: Apoptosis detection,an overview. *Exp Gerontol* 33, 525-533 (1998)

34. Narula J, P. Pandey, E. Arbustini, N. Haider, N. Narula, F.D. Kolodgie, B. Dal Bello, M.J. Semigran, A. Bielsa-Masdeu, G.W. Dec, S. Israels, M. Ballester, R. Virmani, R. Saxena & S. Kharbanda: Apoptosis in heart failure, Release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyocytes. *Proc Nat Acad Sci* 96, 8144-8149 (1999)

35. Jacobson MD, J.F. Burne & M.C. Raff: Programmed cell death and Bcl-2 protection in the absence of a nucleus. *EMBO J* 13, 1899-1910 (1994)

36. Schulze-Osthoff K, H. Walczak, W. Dröge & P.H. Krammer: Cell nucleus and DNA fragmentation are not required for apoptosis. *J Cell Biol* 127, 15-20 (1994)

37. Nishikawa A. & H. Hayashi: Spatial, temporal and hormonal regulation of programmed muscle death during metamorphosis of the frog Xenopus laevis. *Differentiation* 59, 207-214 (1995)

38. Hoffman E., R.H. Brown & L.M. Kunkel: Dystrophin, the protein product of the Duchenne muscular dystrophy locus. *Cell* 51, 919-928 (1987)

39. Bulfield G, W.G. Siller, P.A.L. Wight & K.J: Moore. X-chromosome linked muscular dystrophy (mdx) in the mouse. *Proc Nat Acad Sci* 81, 1189-1192 (1984)

40. Carnwath JW & D.M. Shotton: Muscular dystrophy in the mdx mouse, histopathology of the soleus and extensor digitorum longus muscle. *J Neurol Sci* 80, 39-54 (1987)

41. Anderson JE, B.H. Bressler & W.K. Ovalle: Functional regeneration in the hindlimb skeletal muscle of the mdx mouse. *J Musc Res Cell Motil* 9, 499-515 (1988)

42. Cullen MJ & E. Jaros: Ultrastructure of the skeletalmuscle in the X chromosome-linked dystrophic (mdx) mouse. Comparison with Duchenne muscular dystrophy. *Acta Neuropathol* 77, 69-81 (1988)

43. Stedman HH, H.L. Sweeney, J.B. Shrager, H.C. Maguie, R.A. Panettiere, B. Petrof, M. Narusawa, J.M. Leferovich, J.T. Sladky & A.M. Kelly: The mdx mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy. *Nature* 352, 536-539 (1991)

44. Matsuda R, A. Nishikawa & H. Tanaka: Visualization of dystrophic muscle fibers in mdx mouse by vital staining with Evans blue, evidence of apoptosis in dystrophin-deficient muscle. *J Biochem* 118, 959-964 (1995)

45. Spencer MJ, C.M. Walsh, K.A. Dorshkind, E.M. Rodrigues & J.G. Tidball: Myonuclear apoptosis in dystrophic mdx mouse occurs by perforin-mediated cytotoxicity. *J Clin Invest* 99, 2745-2751 (1997)

46. Sandri M, M. Podhorska-Okolow, V. Geromel, C. Rizzi, P. Arslan, C. Franceschi & U. Carraro: Exercise induces myonuclear ubiquitination and apoptosis in dystrophin-deficient muscle of mice. *J Neuropathol Exp Neurol* 56(1), 45-57 (1997)

47. Sandri M, C. Minetti, M. Pedemonte & U. Carraro: Apoptotic myonuclei in human Duchenne Muscular Dystrophy. *Lab Invest* 78, 1005-1016 (1998)

48. Sandri M, M.L. Massimino, M. Cantini, E. Giurisato, C. Sandri, P. Arslan & U. Carraro: Dystrophin deficient myotubes undergo apoptosis in mouse primary muscle cell culture after DNA damage. *Neurosci Lett* 252(2), 123-126 (1998)

49. Tews DS & H.H. Goebel: DNA-fragmentation and expression of apoptosis-related proteins in muscular dystrophies. *Neuropathol Appl Neurobiol* 23, 331-338 (1997)

50. Olivé M, J.A. Martinez-Matos, J. Montero & I. Ferrer: Apoptosis is not the mechanism of cell death of muscle fibers in human muscular dystrophies and inflammatory myopathies. *Muscle Nerve* 20, 1328-1330 (1997)

51. Inukai A, Y. Kobayashi, K. Ito, M. Doyu, A. Takano, H. Honda & G. Sobue: Expression of FAS antigen is not associated with apoptosis in human myopathies. *Muscle Nerve* 20, 702-709, (1997)

52. Roy RR, K.M. Baldwin & V.R. Edgerton: The plasticity of skeletal muscle, effects of neuromuscular activity. *Exerc Sport Sci Rev* 19, 269-312 (1991)

53. Howard G, J.M. Steffen & T.E. Geoghegan: Transcriptional regulation of decreased protein synthesis during skeletal muscle unloading *J Appl Physiol* 66, 1093-1098 (1989)

54. Thomason DB, P.R. Morrison, V. Oganov, E. Ilyina-Kakueva, F.W. Booth & K.M. Baldwin: Altered actin and myosin expression in muscle during exposure ti microgravity. *J Appl Physiol* 73, Suppl., S90-S93 (1992)

55. Allen DG, S.R. Monke, R.J. Talmadge, R.R. Roy & V.R. Edgerton: Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibres. *J Appl Physiol* 78, 1969-1976 (1995)

56. Allen DG, W. Yasui, T. Tanaka, Y. Ohira, S. Nagoaka, C. Sekiguchi, W.E. Hinds, R.R. Roy & V.R. Edgerton. Myonuclear number and myosin heavy chain expression in rat soleus muscle fibres following spaceflight. *J Appl Physiol* 81, 145-151 (1996)

57. Hikida RS, S. van Nostran, J.D. Murray, R.S. Staron, S.E. Gordon & W.J. Kraemer: Myonuclear loss in atrophied soleus muscle fibres. *Anat Rec* 247, 350-354 (1997)

58. Kerr JFR: Shrinkage necrosis, a distinct mode of cellular death. *J Pathol* 105, 13-20 (1971)

59. Lee LE & R. Altschel: Electron microskopy of the nuclei of denervated skeletal muscle. *Z Zellforsch Mikrosk Anat* 61, 168-182 (1963)

60. Allen DG, J.K. Linderman, R.R. Roy, A.L. Bigbee, R.E. Grindeland, V. Mukku & V.R. Edgerton: Apoptosis, a mechanism contributing to remodeling of skeletal muscle in response to hindlimb unweighting. *Am J Physiol* 273, C579-C587 (1997)

61. McCall GE, C. Goulet, R.E. Grindeland, J.A. Hodgson, A.J. Bigbee & V.R. Edgerton: Bed rest suppresses bioassayable growth hormone release in response to muscle activity. *J Appl Physiol* 83, 2086-2090 (1997)

62. Gobe G, D. Willgoss, D. Hogg, E. Schoch & Z. Endre: Cell survival or death in renal tubular epitheliumafter ischemia-reperfusion injury. *Kidney International* 56, 1299-1304 (1999)

63. Horie Y, R. Wolf & D.N. Granger: Role of nitric oxide in gut ischemia-reperfusion-induced hepatic microvascular dysfunktion. *Am J Physiol* 273, G1007-G1013 (1997)

64. Bielawska AE, J.P. Shapiro, L. Jiang, H.S. Melkonyan, C. Piot, C.L. Wolfe, L.D. Tomei, Y.A. Hannun & S.R. Umansky: Ceramide is involved in triggering of cardiomyocyte apoptosis induced by ischemia and reperfusion. *Am J Pathol* 151, 1257-1263 (1997)

65. Yue TL, X.L. Ma, X. Wang, A.M. Romanic, G.L. Liu, C. Louden, J.L. Gu, S. Kumar, G. Poste, R.R. Ruffolo & G.Z. Feuerstein: Possible involvement of stress-activated protein kinase signaling pathway and FAS receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by carvedilol. *Circ Res* 82, 166-174 (1998)

66. Hachiya J & H. Kazui: Studies of histological and molecular biological changes after graded periods of ischemia-reperfusion in mouse skeletal muscle. *Basic Appl Myol* 6, 302(A) (1996)

67. Knight KR, A. Messina, J.V. Hurley, B. Zhang, W.A. Morrison & A.G. Stewart: Muscle cells become necrotic rather than apoptotic during reperfusion of ischaemic skeletal muscle. *Int J Exp Path* 80, 169-175 (1999)

68. Szlachcic J, B.M. Massie, B.L. Kramer, N. Topic & J. Tubau: Correlates and prognostic implication of exercise capacity in chronic heart failure. *Am J Cardiol* 55, 1037-1042 (1985)

69. Higginbotham MB, K.G. Morris, E.H. Conn, R.E. Coleman & F.R. Cobb: Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardio.* 51, 52-60 (1983)

70. Vescovo G, R. Zennaro, M. Sandri, U. Carraro, C. Leprotti, C. Ceconi, G.B. Ambrosio & L. Dalla-Libera: Apoptosis of skeletal muscle myofibers and interstitial cells in experimental heart failure. *J Mol Cell Cardiol* 30, 2449-2459 (1998)

71. Libera LD, R. Zennaro, M. Sandri, G.B. Ambrosio & G. Vescovo: Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. *Am J Physiol* 277, C982-C986 (1999)

72. Cohen RA, J.T. Shepherd & P.M. Vanhoutte: Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. *Science* 221, 273-274 (1983)

73. Adams V, H. Jiang, J. Yu, S. Möbius-Winkler, E. Fiehn, A. Linke, C. Weigl, G. Schuler & R. Hambrecht. Apoptosis in skeletal myocytes of patients with chronic heart failure is associated with exercise intolerance. *J Am Coll Cardiol* 33, 959-965 (1999)

74. Thomas HC, M.W. Lame, S.K. Dunston, H.J. Segall & D.W. Wilson: Monocrotaline pyrrole induces apoptosis in pulmonary artery endothelial cells. *Toxicol Appl Pharmacol* 151, 236-244 (1998)

75. Howie SEM, A. Sommerfiled, E. Gray & D.J. Harrison: Peripheral T lymphocyte depletion by apoptosis after CD4 ligation in vivo, selective loss of CD44- and 'activating' memory T cells. *Clin Exp Immunol* 95, 195-200 (1994)

76. Tews DS, H.H. Goebel, I. Schneider, A. Gunkel, E. Stennert & W.F. Neiss: DNA-fragmentation and expression of apoptosis-related proteins in experimentally denervated and reinnervated rat facial muscle. *Neuropathol Appl Neurol* 23, 141-149 (1997)

77. Landing BH, L.G. Dixon & T.R. Wells: Studies on isolated human skeletal muscle fibres. Including a proposed pattern of nuclear distribution and a concept of nuclear territories. *Hum Pathol* 5, 441-461 (1974)

78. Ralston E & Z.W. Hall: Restricted distribution of mRNA produced from a single nucleus in hybrid myotubes. *J Cell Biol* 119, 1063-1068 (1992)

79. Dipasquale B & R.J. Youle: Programmed cell death in heterokaryons. A study of the transfer of apoptosis between nuclei. *Am J Pathol* 141, 1471-1479 (1992)

80. Ju ST, K. Matsui & M. Ozdemirli: Molecular and cellular mechanisms regulating T and B cell apoptosis through Fas/FasL interaction. *Int Rev Immunol* 18, 485-513 (1999)

81. Tsubata T: Apoptosis of mature B cells. *Int Rev Immunol* 18, 347-365 (1999)

82. Zhivotosky B, D.H. Burgess, D.M. Vanags & S. Orrenius: Involvement of cellular proteolytic machinery in apoptosis. *Biochem Biophys Res Commun* 230, 481-488 (1997)

83. Yasuhara S, E. Kanakubo, M.E. Perez, M. Kaneki, T. Fujita, T. Okamoto & J.A. Martyn: The 1999 Moyer award. Burn injury induces skeletal muscle apoptosis and the activation of caspase pathway in rats. *J Burn Care Rehabil* 20, 462-470 (1999)

84. Mukasa T, T. Momoi & M.Y. Momoi: Activation of caspase-3 apoptotic pathway in skeletal muscle fibers in laminin alpha2-deficient mice. *Biochem Biophys Res Commun* 260, 139-142 (1999)

85. Takahashi A: Caspase, executioner and undertaker of apoptosis. *Int J Hematol* 70, 226-232 (1999)

86. Budihardjo I, H. Oliver, M. Lutter, X. Luo & X. Wang: Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 15, 269-290 (1999)

87. Kroemer G: The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nature Medicine* 3, 614-620 (1997)

88. Kluck RM, E. Bossy-Wetzel, D.R. Green & D.D. Newmeyer: The release of cytochrome c from mitochondria, a primary site for bcl-2 regulation of apoptosis. *Science* 275, 1132-1136 (1997)

89. Zamzami N, S.A. Susin, P. Marchetti, T. Hirsch, I. Gomez-Monterrey, M. Castedo & G. Kroemer: Mitochondrial control of nuclear apoptosis. *J Exp Med* 183, 1533-1544 (1996)

90. Susin SA, N. Zamzami, M. Castedo, T. Hirsch, P. Marchetti, A. Macho, E. Daugas, M. Geuskens & G. Kroemer: Bcl-2inhibits themirochondrial release of an apoptogenic protease. *J Exp Med* 184, 1331-1342 (1996)

91. Liu X, C.N. Kim, J. Yang, R. Jemmerson & X. Wang: Induction of apoptotic program in cell free extracts, requirement for dATP and cytochrome c. *Cell* 86, 147-157 (1996)

92. Zou H, W.J. Henzel, X. Liu, G. Lutsch & X. Wang: Apaf-1 a human protein homologous to C.elegans CED-4, participates in cytochrom c-dependent activation of caspase-3. *Cell* 90, 405-413 (1997)

93. Zou H, Y. Li, X. Liu & X. Wang: An APAF-1 cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. *J Biol Chem* 274, 11549-11556 (1999)

94. Yuan JY & H.R. Horvitz: The caenorhabditis elegans genes ced-3 and ced-4 act cell autonomously to cause programmed cell death. *Dev Biol* 138, 33-41 (1990)

95. Li P, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri & X. Wang: Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 19, 479-489 (1997)

96. Yang, J., X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones & X. Wang: Prevention of apoptosis by bcl-2, release of cytochrom c from mitochondria blocked. *Science* 275, 1129-1132 (1997)

97. Fehsel K, K.L. Kröncke, K.L. Meyer, H. Huber, V. Wahn & V. Kolb-Bachofen: Nitric oxide induces apoptosis in mouse thymocytes. *J.Immunol* 155, 2858-2865 (1995)

98. Messmer UK, E.G. Lapetina & B. Brüne: Nitric oxide-induced apoptosis in RAW 264.7 macrophages is antagonized by protein kinase C - and protein kinase A - activating compounds. *Mol Pharmacol* 47, 757-765 (1996)

99. Arstall MA, D.B. Sawyer, R. Fukazawa & R.A. Kelly: Cytokine-mediated apoptosis in cardiac myocytes. The role of inducible nitric oxide synthase induction and peroxynitrite generation. *Circ Res* 85, 829-840 (1999)

100. Stangel M, U.K. Zettl, E. Mix, J. Zielasek, K.V. Toyka, H.P. Hartung & R. Golg: H2O2 and nitric oxide-mediated oxidative stress induces apoptosis inrat skeletal muscle myoblasts. *J Neuropathol Exp Neurol* 55, 36-43 (1996)

101. Adams V, J. Yu, S. Möbius-Winkler, A. Linke, C. Weigl, L. Hilbrich, G. Schuler & R. Hambrecht: Increased nitric oxide synthase in skeletal muscle biopsies from patients with chronic heart failure. *Biochem Mol Med* 61, 152-160 (1997)

102. Riede U, U. Förstermann & H. Drexler: Inducible nitric oxide synthase in skeletal muscle of patients with chronic heart failure. *J Am Coll Cardiol* 32, 964-969 (1998)

103. Hambrecht R, V. Adams, S. Gielen, A. Linke, S. Möbius-Winkler, J. Yu, J. Niebauer, H. Jiang, E. Fiehn & G. Schuler: Exercise intolerance in patients with chronic heart failure and increased expression of inducible nitric oxide synthase in the skeletal muscle. *J Am Coll Cardiol* 33, 174-179 (1999)

104. Hortelano S, A.M. Alvarez & L. Bosca: Nitric oxide induces tyrosin nitration and release of cytochrome c preceding an increase of mitochondrial transmembrane potential in macrophages. *FASEB J* 13, 2311-2317 (1999)

Abbreviations: ARC : apoptosis repressor with caspase recruitment domain, CHF : chronic heart failure, DMD : Duchenne muscular dystrophy , FACS : flouresence activated cell sorter, NO : nitric oxide, PARP : poly-ADP-ribose polymerase, PCD : programmed cell death, TUNEL : terminal deoxynucleotidyl transferase mediated dUTP nick end labeling

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