Characterization of canine mitochondrial protein expression in natural and induced forms of idiopathic dilated cardiomyopathy

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Objective—To map canine mitochondrial proteins and identify qualitative and quantitative differences in heart mitochondrial protein expression between healthy dogs and dogs with naturally occurring and induced dilated cardiomyopathy (DCM).

Sample Population—Left ventricle samples were obtained from 7 healthy dogs, 7 Doberman Pinschers with naturally occurring DCM, and 7 dogs with induced DCM.

Procedures—Fresh and frozen mitochondrial fractions were isolated from the left ventricular free wall and analyzed by 2-dimensional electrophoresis.

Results—Within narrow pH gradients of control canine heart mitochondrial samples, a total of 1,528 protein spots were revealed. Forty subunits of heart mitochondrial proteins that differ significantly from control tissues were altered in tissue specimens from dogs with naturally occurring and induced forms of DCM. The most affected heart mitochondrial proteins in both groups were those of oxidative phosphorylation (85%). Upregulation of manganese superoxide dismutase was suggestive of heart oxidative injury in tissue specimens from dogs with both forms of DCM. Evidence of apoptosis was associated with overexpression of the heart mitochondrial voltage-dependent anion channel-2 protein and endonuclease G in tissue specimens from dogs with induced DCM.

Conclusions and Clinical Relevance—Alterations of heart mitochondrial proteins related to oxidative phosphorylation dysfunction were more prevalent in tissue specimens from dogs with induced or naturally occurring DCM, compared with those of control dogs. (Am J Vet Res 2006;67:963–970)

ABBREVIATIONS

DCM Dilated cardiomyopathy
TCA Tricarboxylic acid
pI Isoelectric point
MALDI-TOF Matrix-assisted laser desorption/ionization time-of-flight
QqTOF Quadrupole selecting, quadrupole collision cell, time-of-flight mass spectrometry
OAT Ornithine aminotransferase
MnSOD Manganese superoxide dismutase
ALR Augmenter of liver regeneration
STAR Steroidogenic acute regulatory
CA Carbonic anhydrase
COX 3-hexaprenyl-4, 5-dihydroxybenzoate methyltransferase
TIM22 Inner membrane translocase 22
VADC-2 Voltage-dependent anion channel-2

It is believed that mitochondria are intimately involved in the pathogenesis of heart failure. Several human disorders are linked to mitochondrial dysfunction, and the most affected tissues are those that have a high demand for energy such as heart, brain, muscle, and kidney. Even though mitochondrial diseases are well known in humans, little is known about mitochondrial function in canine myocardial diseases. In the heart, mitochondria are mainly involved in energy production. Mitochondria also participate in cellular homeostasis, signaling, metabolism, and apoptosis.

The canine mitochondrial genome is a circular, double-stranded molecule of 16,728 bp. Mitochondrial DNA encodes for 2 ribosomal RNAs (12S and 16S), 22 transfer RNAs, and 13 subunits of the respiratory chain. Approximately 90 protein subunits of the respiratory chain complex exist, of which only 13 subunits are encoded by mitochondrial DNA. However, mitochondrial DNA subunits are disproportionately represented because of the multiplicity of mitochondria copies per cell. In humans, it is estimated that approximately 1,000 to 2,000 mitochondrial proteins exist. When researching the pathophysiology of mechanisms responsible for altered mitochondrial function in DCM, proteomics offers several advantages over genomic techniques, such as the ability to detect the presence of isoforms and posttranslational modifications in cells or tissue and to directly quantify protein expression. This may be important in studies of mitochondria, as this organelle has been shown to be altered by fluctuating protein turnover and release as...
well as by transport of proteins into mitochondria from the cytosol.

Dilated cardiomyopathy is a myocardial disease characterized by ventricular dilation and reduced cardiac function. Several causes have been implicated in DCM involving different metabolic and signaling pathways. Dogs with rapid ventricular pacing have been used to study the pathophysiologic changes involved in DCM and congestive heart failure. Dogs with experimentally induced rapid ventricular pacing have compromised cardiac function that induces myopathy leading to increased chamber dilation and ventricular stress. To our knowledge, a comparison of canine mitochondria protein expression to determine how well the induced form of DCM mimics the naturally occurring disease has not been done.

On the basis of what is known from studies in various species, we anticipate that energy metabolism related to oxidative phosphorylation, TCA cycle, and proteins associated with programmed cell death will be altered protein expression. The purposes of the study reported here were to map the canine mitochondrial proteins and identify qualitative and quantitative differences in heart mitochondrial protein expression between healthy dogs and dogs with naturally occurring and induced DCM. Our hypotheses are that qualitative and quantitative differences in mitochondrial protein expression from the left ventricle will be found between control dogs and dogs with naturally occurring or induced forms of DCM. In this study, canine mitochondrial proteins from the left ventricle were sampled and analyzed to compare mitochondrial protein expression between tissue specimens from healthy dogs, Doberman Pinschers with naturally occurring DCM, and dogs subjected to rapid ventricular pacing.

Materials and Methods

Collection of tissue specimens—Left ventricular free wall was obtained from 7 clinically healthy mixed-breed dogs immediately after euthanasia. These tissues served as controls for all subsequent studies. Dogs were part of a terminal study unrelated to this project and ranged in age and weight from 4 to 8 years old and 20 to 35 kg, respectively. Left ventricular free wall was also obtained from 7 Doberman Pinschers with spontaneously occurring DCM at the time of euthanasia. All dogs in this group were at the end stage of disease with mean percent fractional shortening of 13.4%. The age and body weight of this group ranged from 4 to 11.5 years old and 29.5 to 48 kg, respectively.

The experimental group dogs included 7 adult male hounds weighing between 21 to 35 kg. Three dogs were subjected to rapid ventricular pacing by use of an endocardial lead system placed in the apex of the right ventricle. The other 4 dogs were induced to rapid ventricular pacing by use of an epicardial lead system placed in the apex of the right ventricle. Initially, dogs were paced at 180 beats/min for 10 days. Subsequently, the pacing rates were increased to 200, 210, 220, and 240 beats/min, respectively in 4 days. In this group, the mean percent fractional shortening decreased from 30.5% to 12.5% at the time of euthanasia. Left ventricular free wall was collected from all dogs. The heart muscle specimens were either stored at –70°C in liquid nitrogen immediately after euthanasia or used immediately for mitochondrial fractionation. All dogs in this study were euthanized by IV administration of a standard pentobarbital-based euthanasia solution.

Mitochondrial isolation—Heart tissues were cleaned of connective tissue and fat and minced into 0.2- to 0.3-mm-wide pieces. One hundred-milligram aliquots were homogenized in ice-cold mitochondrial isolation buffer with a dounce tissue grinder, transferred to 1.5-mL microcentrifuge tubes, and centrifuged at 600 X g for 5 minutes. The supernatant was transferred to new microcentrifuge tubes and centrifuged at 11,000 X g for 10 minutes. The mitochondria pellets were collected. The total protein was measured by use of bicinchoninic acid with bovine serum albumin standards, and pellets were stored in liquid nitrogen.

Two-dimensional gel electrophoresis and imaging analysis — The 2-dimensional electrophoresis protocol included the use of a commercial system and pI 3 to 10 linear immobilized pH gradient strips for isoelectric focusing. Polyacrylamide electrophoresis gels (11 cm in length; 12%) were used for second dimension electrophoresis, allowing up to 12 gels to run simultaneously. Each mitochondria sample was run in triplicate, applying 100 µg of protein sample to each gel, along with 2-dimensional gel electrophoresis standards. All gels were stained with silver. Gels were scanned and resulting protein expression patterns analyzed with 2-dimensional gel analysis software. Molecular mass and pI values were automatically calculated for the remaining protein spots. The quantitative analysis of protein spots was determined. Decreases or increases of 2-fold or greater of mitochondrial protein expression in tissue specimens from diseased dogs, compared with control dogs, were considered substantial and evaluated. Statistical analysis was performed to compare mitochondrial protein expression between tissue specimens of control and diseased dog groups by use of a Mann-Whitney test. Significance was set at a value of P < 0.05. All mitochondrial protein spots found to differ significantly and by at least 2-fold were visually matched to confirm differences in their protein expression from control dogs. Mitochondrial protein samples from 3 control dogs were run in triplicate within narrow pH gradients of 3 to 6, 6 to 8, and 7 to 10 by use of the protocol already described.

Trypsin digestion and analysis by mass spectrometry — Only spots that differed significantly between control and diseased dog groups and by at least 2-fold were selected for mass spectrometry analysis. Protein in-gel digestion for each selected spot was processed after manual protein spot excision with a 1.3-mm-diameter spot cutter into a silica-based microcentrifuge tube. Each selected spot was visually assessed to compare the altered mitochondrial protein expression between control and diseased dog groups. A protein in-gel digestion kit was used for digestion of protein spots according to recommendations of the manufacturer. Prepared samples were analyzed by MALDI-TOF mass spectrometry with the use of a biospectrometry workstation. External calibration of the biospectrometry workstation was performed with human angiotensin I that had a mass average of 1,297.50 daltons and bovine insulin that had a mass average of 5,734.60 daltons.

Each protein spot was run 3 times in the MALDI-TOF spectrometer. Mass spectral data for each protein were compared with protein databases to achieve protein identification. For protein identification, 3 Web sites were searched. The monoisotopic peptide masses were searched in a protein database. A peptide mass tolerance of 1 dalton was allowed for all Web site databases. Mitochondrial protein spots not identified by MALDI-TOF mass spectrometry were analyzed by QqTOF mass spectrometry with an orthogonal accelerator.
Results

Within the narrow pH gradients of canine heart mitochondrial samples (which ranged between 3 to 6, 5 to 8, and 7 to 10) a total of 1,528 protein spots were revealed. Mitochondrial protein spots had a molecular mass and pI that ranged between 10.0 to 110 kd and 3.0 to 10.0, respectively. Most mitochondrial proteins were concentrated in a molecular mass range of 20 to 40 kd and pI range of 5.87 to 6.68 (Figures 1 and 2). For tissue specimens from each dog, triplicate gels were run resulting in a mean ± SD number for matched mitochondrial protein spots in tissue specimens of 345 ± 29 for control dogs, 355 ± 29 for dogs with naturally occurring DCM, and 356 ± 28 for dogs with induced DCM. Within each group, no significant qualitative changes in mitochondrial protein expression among gels or dogs or between matched fresh and frozen heart tissues were found. However, significant differences in protein expression were found among tissue specimens from each of the 3 groups. Expression analysis revealed 40 protein spots in tissue specimens from control dogs with a mean expression value that was significantly different by at least 2-fold in tissue specimens from dogs with induced or naturally occurring DCM. Analysis by MALDI-TOF mass spectrometry was performed on the 40 protein spots. Six of 40 mitochondrial protein spots were further analyzed by QqTOF mass spectrometry to obtain their identity. The distribution of molecular mass and pI values for these altered protein spots ranged from 13.79 to 102.09 kd and 4.2 to 8.31, respectively. Altered heart mitochondrial protein spots were mainly related with oxidative phosphorylation (55%), energy (15%), metabolism (12.5%), DNA and RNA protein synthesis (7.5%), protein targeting (5%), transport (2.5%), and signaling (2.5%). Tissue specimens from dogs with induced or naturally occurring DCM had a proportional number of altered mitochondrial protein spots according to function but different identity and protein expression patterns. Three of 6 mitochondrial protein spots involved in primary energy function were found in tissue specimens from dogs with induced DCM and dogs with naturally occurring DCM; in dogs with naturally occurring DCM, this included the downregulation of malate dehydrogenase (Figure 3) and cytochrome P450 11B2, which were identified with a match of 3 and 4 peptides and sequence coverage of 17.9% and 13%, respectively. In addition, upregulation of MnSOD was also observed in tissue specimens from dogs with naturally occurring DCM (Table 1). Manganese superoxide dismutase was identified with 1 peptide, 179-AIWNINWENVTER-192, by use of QqTOF mass spectrometry.

The ALR protein, which was downregulated and identified with a match of 3 peptides and 16.9% sequence coverage, was among the altered proteins associated with primary energy in dogs subjected to rapid ventricular pacing. Moreover, 2 isoforms of MnSOD (Figure 4) were upregulated in dogs subjected to rapid ventricular pacing. One MnSOD isoform was identified with 3 peptides and 44% sequence coverage and reassured by QqTOF mass spectrometry (Table 1). The other MnSOD isoform
was identified through QqTOF mass spectrometry analysis. In tissue specimens from dogs with naturally occurring DCM, only 2 proteins associated with metabolism were downregulated. These included pyruvate dehydrogenase E1 α-subunit (Figure 3) and the STAR protein; both proteins were matched with 4 peptides and sequence of coverage of 23.3% and 32.4%, respectively. Three mitochondrial protein spots associated with metabolism were related to the induced form of DCM. Sarcosine dehydrogenase and OAT were overexpressed; both proteins had a match of 4 peptides with 15% and 10.2% sequence coverage, respectively. Carbonic anhydrase was downregulated and identified with 3 matched peptides and 19% sequence coverage. Two mitochondrial proteins associated with protein synthesis were overexpressed in tissue specimens from dogs in both diseased groups. In tissue specimens from dogs with naturally occurring DCM, the protein 50S ribosomal L22 was identified with 3 matched peptides and 34% sequence coverage. Mitochondrial proteins associated to protein synthesis were overexpressed in tissue specimens from dogs with naturally occurring DCM. 1 mitochondrial protein spot, COQ3, was upregulated and associated with protein targeting, whereas the A-kinase anchor protein, related to signaling function, was downregulated. Both proteins had a match of 3 peptides and sequence coverage of 20.3% and 8%, respectively. In tissue specimens from dogs subjected to rapid ventricular pacing, 1 protein spot, TIM22, was associated with protein targeting; TIM22 was identified with a match of 3 peptides and sequence of coverage of 23%. The VADC-2 protein is related to transport. The TIM22 and VADC-2 proteins were overexpressed in dogs with induced DCM. Analysis of the VADC-2 protein with QqTOF mass spectrometry revealed the following matching peptides: 27-DIFNRGFGFGLVK-39 (substitution of R for K-5), 108-LFTDTTSPNTGKK-121, 236-YQLDPTASISAK-247, 248-VNNSSLIGV-GYTQTLRPGV-267, and 268-LTLSALVDGK-277. Mass spectrometry identification of mitochondrial proteins was based on mitochondrial protein databases from different species because no complete database of canine mitochondrial proteins exists. The functional classification of the canine heart mitochondrial proteins identified in our study followed the human mito-

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*Online database or National Center of Biotechnology Information accession numbers are shown. Proteins analyzed by MALDI-TOF mass spectrometry. Proteins identified by QqTOF mass spectrometry. Dec = Decreased protein expression by 2-fold or greater. Inc = Increased protein expression by 2-fold or greater. IM = Inner mitochondrial membrane. IMS = Intermembrane space. OM = Outer mitochondrial membrane. DNA = Mitochondrial DNA. Cyt = Cytochrome. NC = No change.
The total number of canine heart mitochondrial proteins mapped by 2-dimensional electrophoresis at narrow pH gradients in this study, 1,528 protein spots, is close to the expected number of human mitochondrial proteins, considering that highly acidic or basic proteins were not analyzed. Some mitochondrial proteins analyzed by MALDI-TOF mass spectrometry had different pIs, compared with their identity match. According to Weiller et al., changes in pI of proteins can be related to different chemical properties of amino acids in different species. Comparing the functional classification, it was observed that more than half of the altered mitochondrial proteins were associated with oxidative phosphorylation function in tissue specimens from dogs with induced or naturally occurring DCM. These findings emphasize the importance of energy deficits in dogs with naturally occurring and induced forms of DCM. However, no difference in mitochondrial protein expression changes was found between tissue specimens from dogs with induced or naturally occurring DCM. One factor that should be considered is that clinically affected dogs with natural forms of DCM in our study were receiving treatment for heart failure, consisting primarily of positive inotropic agents, diuretics, and angiotensin-converting enzyme inhibitors. However, we believe that it is likely that many of the observed differences in mitochondrial protein expression represent inherent differences between clinical and experimentally induced forms of DCM.

In the primary energy function group, half of the mitochondrial proteins identified were related to MnSOD. In tissue specimens from dogs with naturally occurring DCM, 1 mitochondrial protein spot was identified as MnSOD, and in dogs subjected to rapid ventricular pacing, 2 isoforms of MnSOD were observed. The upregulation of MnSOD in both forms of DCM suggests that free radicals are increased, which could be one of the factors inducing oxidative phosphorylation dysfunction. Induction of MnSOD was observed in patients with a poor prognosis from complex I deficiency. Moreover, reactive oxygen species generated by mitochondria have been implicated in oxidative damage of proteins leading to loss or impaired function. Complex I is the main source of free radicals, and free radical damage directly affects its function.

Experiments with superoxide dismutase 2 mutants revealed that impaired function of the reduced form of nicotinamide adenine dinucleotide-oxidoreductases and iron-sulfur proteins were associated with increased oxidative damage. In our study, malate dehydrogenase, 1 of the enzymes of the TCA cycle, was identified by mass spectrometry with sequence coverage of 17.9% with Arabidopsis thaliana. Results of phylogenetic studies indicate that mitochondrial malate dehydrogenase is closely related in different species. In the TCA cycle, malate dehydrogenase produces the reduced form of nicotinamide adenine dinucleotide that is channeled to complex I and is used in electron transfer of electron transport chain. Downregulation of this protein in the natural form of DCM suggests that a decreased source of the reduced form of nicotinamide adenine dinucleotide is being produced, compromising electron transfer. Proteomics studies also found decreased expression of malate dehydrogenase in atrial tissue of human patients with DCM.

Several studies have been designed to evaluate the benefits of pharmaceutical agents related to patient mitochondrial protein classification from a protein data list.
survival when administered alone or in combination. However, effects of drug treatment on mitochondrial protein expression are not well known. Cytochrome P450 11B2, also known as aldosterone synthase, which is responsible for the conversion of 11-deoxy cortisol to aldosterone, was downregulated in tissue specimens from dogs with naturally occurring DCM in our study. This finding may be associated with the use of spironolactone and angiotensin-converting enzyme inhibitors as chronic treatment in DCM. Amounts of cytochrome P450 11B2 mRNA were highly expressed in patients with congestive heart failure and systolic dysfunction. In comparison, lower expression of cytochrome P450 11B2 mRNA was observed in patients under treatment with spironolactone and angiotensin-converting enzyme inhibitors. However, it is not known if such medications induce changes to mitochondrial protein expression.

The ALR protein, which is an ortholog of the erv1-like growth factor protein, is involved in the biogenesis of iron-sulfur clusters of complexes I, II, and III in mitochondria. Downregulation of ALR protein in the induced form of DCM could alter the expression pattern of iron-sulfur proteins. For instance, the downregulation of the 30-kd iron-sulfur protein in complex I and upregulation of succinate dehydrogenase iron-sulfur protein in complex II could be caused by an indirect effect of ALR protein on protein expression or impaired function caused by oxidative damage. Downregulation of mitochondrial proteins may also be caused by posttranslational modifications leading to changes in molecular mass and the pl of proteins.

In the metabolism function group, 2 identified mitochondrial proteins, pyruvate dehydrogenase E1 α-subunit and STAR protein, were downregulated in tissue specimens from dogs with naturally occurring DCM. Pyruvate dehydrogenase is a multienzyme complex composed of E1, E2, and E3 subunits that connect glycolysis from the cytosol to the TCA cycle in the mitochondrial matrix. Phosphorylation-dephosphorylation of E1 α-subunit is responsible for regulation of this multienzyme. Downregulation of pyruvate dehydrogenase E1 α-subunit suggests that this protein has an altered function resulting in a decreased amount of reduced nicotinamide adenine dinucleotide that will be transferred from the TCA cycle to the electron transport chain. In contrast upregulation of pyruvate dehydrogenase E2 β-subunit was observed in dogs with rapid ventricular pacing. The STAR protein is involved in lipid transport and metabolism, signal transduction, and transcription. In prokaryotes, STAR protein is also linked to regulation of fatty-acid metabolism. In myocardial infarction, increased cardiac angiotensin II induces STAR protein mRNA expression, and as a consequence higher amounts of aldosterone are produced. Downregulation of STAR protein in dogs with naturally occurring DCM could be related to the use of spironolactone and angiotensin-converting enzyme inhibitors, as occurred with cytochrome P450 11B2 (aldosterone synthase).

In the induced form of DCM, 2 mitochondrial proteins that are associated with amino acid metabolism (ie, OAT and sarcosine dehydrogenase) were overexpressed. Ornithine aminotransferase converts ornithine to glutamate and is part of the urea cycle. Ornithine also is an important precursor in metabolic pathways including proline, glutamate, glutamine, citrulline, and arginine biosynthesis. Ornithine-derived glutamate is metabolized to glutamine that is the precursor of glutathione. Glutathione peroxidase, SOD, and catalase act as scavenger enzymes preventing cellular damage produced by free radicals. Results of a study on cardiomyocyte viability after ischemia-reperfusion injury indicate that glutamine increases myocardial survival by protecting mitochondrial structure and function from oxidative damage. The overexpression of OAT in dogs subjected to rapid ventricular pacing can be associated with rescue of mitochondrial function through increased amounts of antioxidants fighting against oxidative injury. Sarcosine dehydrogenase converts sarcosine to glycine. In humans, hypersarcosemia was previously primarily considered a benign condition. Reports of myocarditis in patients with DCM have been associated with the presence of an antimitochondrial antibody containing an epitope of bacterial sarcosine dehydrogenase. No evidence of myocardium alterations are associated with sarcosine in dogs subjected to rapid ventricular pacing because no specific histologic evaluation has been performed.

In dogs subjected to rapid ventricular pacing, CA was downregulated. Approximately 14 α-CA isoforms have been found in vertebrates. Mitochondrial CA is part of different biosynthetic processes involving pyruvate carboxylase, carbamoyl phosphate I and II, and bicarbonate. Its main function is the carbon dioxide hydration-dehydration of bicarbonate. Mitochondrial CA provides bicarbonate for the carboxylation of pyruvate forming oxaloacetate. Underexpression of mitochondrial CA is indicative of decreased oxaloacetate formation in the TCA cycle, directly affecting electron transfer in the electron transport chain and compromising ATP synthesis.

In the DNA and RNA protein synthesis group, all proteins were overexpressed and were related to translation or programmed cell death. Mitochondrial ribosomal proteins are responsible for protein synthesis of 13 proteins inside mitochondria. Ribosomal proteins express highly conserved sequences in mitochondria from bacteria to euukaryotes. In our study, 5OS ribosomal protein L22 was identified by MALDI-TOF mass spectrometry in tissue specimens from dogs with naturally occurring DCM with 34% sequence coverage in chloroplast. In dogs subjected to rapid ventricular pacing, 39S ribosomal protein L28 also had altered expression. Overexpression of ribosomal proteins can be related to oxidative phosphorylation dysfunction, which was observed in tissue specimens from dogs with induced or naturally occurring DCM and may reflect rapid protein turnover as a result of degradation or altered function. Endonuclease G translates from the intermembrane space to cleave chromatin DNA in the nucleus during apoptosis. This protein, related to programmed cell death, acts in a caspase-independent apoptotic pathway and was upregulated in dogs subjected to rapid ventricular pacing. According to
Cesseli et al altered heart function in dogs with rapid ventricular pacing is correlated to oxidative damage and cardiac cell death. In our study, altered protein expression in dogs subjected to rapid ventricular pacing resulted in upregulation of MnSOD, probably induced by the presence of free radicals; overexpression of VDAC-2 protein leading to increased mitochondrial membrane permeability; and increased expression of endonuclease G. These findings are evidence of oxidative damage and apoptosis in myocardial tissues of dogs with rapid ventricular pacing.

In the protein-targeting functional group, 2 different mitochondrial proteins were identified in tissue specimens from dogs with induced DCM and dogs with naturally occurring DCM. In dogs subjected to rapid ventricular pacing, TIM22 was upregulated. Inner membrane translocase 22 is responsible for the translocation of proteins that need an internal signal across the inner mitochondrial membrane; TIM22 is also important for biogenesis of ATP-ADP carrier, TIM 22 23, and itself.49,50 Upregulation of TIM22 suggests that an increased number of preproteins are being translocated to the inner mitochondrial membrane or that TIM22 is not being properly assembled.

Obligatory in the biosynthesis of ubiquinone, COQ10, was upregulated in tissue specimens from dogs with naturally occurring DCM. This protein is found in the inner mitochondrial membrane and matrix.50 Overexpression of this protein can be associated with energy deficits because ubiquinone is a crucial component of electron transfer in oxidative phosphorylation.

In the transport function group, only VADC-2 protein had altered expression in dogs subjected to rapid ventricular pacing. The voltage-dependent anion channel proteins are integral membrane proteins present in the outer mitochondrial membrane and are involved in apoptosis through increased mitochondrial membrane permeability.51 Upregulation of VADC-2 protein implies that permeability transition in the outer mitochondrial membrane is increased, favoring the release of anions and apoptotic factors.

The A-kinase anchor protein, related to signaling function, was downregulated in tissue specimens from dogs with naturally occurring DCM. This protein has different catalytic subunits that are assembled to make different isoforms.52 Isoform AKAP121 phosphorylates the Bcl-xL/Bcl-2–associated death promoter in the cytoplasmic side of mitochondria, thereby inhibiting cytochrome c release and preventing apoptosis.53 Downregulation of A-kinase anchor protein may be suggestive of reduced mitochondrial protection against apoptotic factors, intense protein turnover, or altered function as a result of posttranslational modifications.

References


