Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy

Emma J. Birks†, Najma Latif†, Karine Enesa2, Tonje Folkvang2, Le Anh Luong2, Padmini Sarathchandra1, Mak Khan1, Huib Ovaa3, Cesare M. Terracciano1, Paul J.R. Barton1, Magdi H. Yacoub1, and Paul C. Evans2*

1Heart Science Centre, National Heart and Lung Institute, Imperial College London, Harefield Hospital, Harefield, UK; 2British Heart Foundation Cardiovascular Sciences Unit, National Heart and Lung Institute, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 ONN, UK; and 3Department of Cellular Biochemistry, Netherlands Cancer Institute, Amsterdam, The Netherlands

Received 20 August 2007; revised 28 February 2008; accepted 19 March 2008; online publish-ahead-of-print 28 March 2008

Time for primary review: 22 days

Aims The molecular mechanisms that regulate cardiomyocyte apoptosis and their role in human heart failure (HF) are uncertain. Expression of the apoptosis regulator p53 is governed by minute double minute 2 (MDM2), an E3 enzyme that targets p53 for ubiquitination and proteasomal processing, and by the deubiquitinating enzyme, herpesvirus-associated ubiquitin-specific protease (HAUSP), which rescues p53 by removing ubiquitin chains from it. Here, we examined whether elevated expression of p53 was associated with dysregulation of ubiquitin-proteasome system (UPS) components and activation of downstream effectors of apoptosis in human dilated cardiomyopathy (DCM).

Methods and results Left ventricular myocardial samples were obtained from patients with DCM (n = 12) or from non-failing (donor) hearts (n = 17). Western blotting and immunohistochemistry revealed that DCM tissues contained elevated levels of p53 and its regulators MDM2 and HAUSP (all P < 0.01) compared with non-failing hearts. DCM tissues also contained elevated levels of polyubiquitinated proteins and possessed enhanced 20S-proteasome chymotrypsin-like activities (P < 0.04) as measured in vitro using a fluorogenic substrate. DCM tissues contained activated caspases-9 and -3 (P < 0.001) and reduced expression of the caspase substrate PARP-1 (P < 0.05). Western blotting and immunohistochemistry revealed that DCM tissues contained elevated expression levels of caspase-3-activated DNAse (CAD; P < 0.001), which is a key effector of DNA fragmentation in apoptosis and also contained elevated expression of a potent inhibitor of CAD (ICAD-S; P < 0.01).

Conclusion Expression of p53 in human DCM is associated with dysregulation of UPS components, which are known to regulate p53 stability. Elevated p53 expression and caspase activation in DCM was not associated with activation of both CAD and its inhibitor, ICAD-S. Our findings are consistent with the concept that apoptosis may be interrupted and therefore potentially reversible in human HF.

KEYWORDS Dilated cardiomyopathy; Apoptosis; p53; Ubiquitin-proteasome system; Caspases

1. Introduction

Human heart failure (HF) is associated with elevated expression of p53,1,2 a transcription factor that induces pro-apoptotic molecules (e.g. Bax) and activates caspases in cardiomyocytes.3 In addition, animal studies have revealed that elevated p53 levels accompany cardiac hypertrophy in response to pressure overload,4–6 HF induced by pacing,7 and HF in mice lacking the telomerase gene.8 A role for p53 in the pathogenesis of HF has been revealed in a recent study in which genetic deletion of p53 protected murine myocardium from injury and enhanced angiogenesis and cardiac function in response to chronic pressure overload.9 Taken together these studies highlight the potential importance of p53 in regulating cardiomyocyte viability and function in HF.

In addition to elevated p53 expression, cardiomyocytes in failing hearts display other molecular changes that are characteristic of apoptosis including activation of caspases and cytochrome c release from mitochondria.9–11 Caspases are cysteine proteases that play a central role in apoptosis by activating several downstream effectors, e.g. they regulate caspase-3-activated DNAase [CAD; otherwise termed as

1 The first two authors made equal contributions.
* Corresponding author. Tel: +44 20 838 31619; fax: +44 20 838 31640.
E-mail address: paul.evans@imperial.ac.uk

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2008.
For permissions please email: journals.permissions@oxfordjournals.org.
DNA fragmentation factor (DFF) by cleaving its inhibitor, ICAD.\textsuperscript{12,13} The potential role of caspase activation in HF has been demonstrated using transgenic mice in which caspase activation led to cardiomyocyte apoptosis and the development of HF.\textsuperscript{14,15} However, the role of caspase activation and apoptosis in regulating cardiac remodelling in human HF remains uncertain.\textsuperscript{16–18} Several studies have revealed that caspase activation in human HF is not always accompanied by other hallmarks of apoptosis including DNA fragmentation, chromatin condensation, and changes in nuclear morphology.\textsuperscript{19–21} In addition, it has been suggested that DNA fragmentation occurring during cardiac hypertrophy can be repaired.\textsuperscript{22,23} Thus, it has been suggested that caspase activation in HF may not lead to activation of downstream executioners of apoptosis, a physiological state that is termed as ‘interrupted apoptosis’.\textsuperscript{20}

The ubiquitin-proteasome system (UPS) protects cardiomyocytes by preventing the build-up of misfolded proteins and by removing pro-apoptotic signalling molecules. Ubiquitin is covalently attached to lysine residues of cellular proteins, through a chain reaction controlled by E3 proteins which contain binding sites for particular cellular proteins.\textsuperscript{24} Ubiquitinated proteins are typically degraded by the 26S-proteasome which is a large multi-protein complex comprising two 19S regulatory particles that contain binding sites for ubiquitinated proteins and a 20S catalytic core that contains peptidases with chymotrypsin- and trypsin-like activities.\textsuperscript{24} Thus, proteasome inhibitors interfere with homeostasis of cultured cardiomyocytes by allowing p53 and other pro-apoptotic proteins to accumulate to high levels and trigger apoptosis. Recent studies have revealed that ubiquitination can be reversed by deubiquitinating cysteine proteases, which regulate the stability or activity of specific cellular proteins by cleaving ubiquitin from them.\textsuperscript{24} Indeed, the stability of p53 relies on a complex interplay between E3 ligases, e.g. minute double minute 2 (MDM2) which attach polyubiquitin chains to p53 and deubiquitinating enzymes, e.g. herpesvirus-associated ubiquitin-specific protease (HAUSP) which can remove them.\textsuperscript{25–28}

Here, we report that elevated expression of p53 in human dilated cardiomyopathy (DCM) is associated with dysregulation of UPS components that are known to regulate p53 stability. Elevated p53 expression in DCM was associated with caspase activation and elevated expression of the downstream executioner of apoptosis CAD. Interestingly, we also observed elevated levels of a spliced form of its inhibitor ICAD-S in DCM, which may interrupt apoptosis by suppressing DNA fragmentation.

\section*{2. Methods}

\subsection*{2.1 Patients and tissue samples}

The HF group consisted of 12 patients undergoing heart transplantation for DCM. All patients underwent a prior assessment that included a medical history, clinical examination, two-dimensional echocardiography, cardiac catheterization, evaluation of haodynamic function, and coronary arteriography. Details of the patients and their haodynamic parameters are shown in Table 1. The control group of non-failing hearts were obtained from 17 donors used for transplantation (11 male, six female; mean age 33.5, age range 2–51 years). Donor hearts were assessed using anti-HA epitope antibodies (1:1000; Roche, Switzerland), and chemiluminescent detection. Protein loading was normalized by western blotting using anti-GAPDH antibodies (Santa Cruz Biotechnology). Expression levels of particular proteins were quantified by laser densitometry of specific bands on autoradiographs and standardized to total protein levels in each respective lane. Densitometric analysis was carried out using the QUANT ONE software (Biorad, USA).

\begin{table}
\centering
\caption{Demographics of patients}
\begin{tabular}{|l|}
\hline
DCM ($n = 12$) \\
\hline
Male:Female & 10:2 \\
Mean age (range) & 42.4 (22–64) \\
Mean LVEDD (mm) & 70.9 (59–90.9) \\
Mean LVESD (mm) & 62.5 (50–81.8) \\
Mean fractional shortening & 13% (6–20%) \\
Mean ejection fraction & 21% (11–38%) \\
NYHA Class 3:4 & 10:2 \\
Diuretics & 10 \\
ACE-inhibitors & 9 \\
Inotropes & 6 \\
Digoxin & 4 \\
Nitrates & 2 \\
\beta-Blockers & 2 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Causes of death}
\begin{tabular}{|l|}
\hline
\textit{Dilated cardiomyopathy (DCM)} & \textit{n = 6}, road traffic accident ($n = 5$), intracranial bleed ($n = 4$), asthma ($n = 1$), and one was a domino heart from a patient undergoing heart-lung transplantation for cystic fibrosis. Hearts were reassessed at one week after transplantation and all had good ventricular function (mean EF 71.8 ± 1.4%). Ventricular myocardial specimens were obtained by endomyocardial biopsy immediately prior to transplantation from the 17 non-failing donor hearts and snap-frozen using liquid nitrogen prior to storage at −80°C. The protocol was approved by the Hillingdon Health Authority Ethics Committee and procedures followed were in accordance with institutional guidelines and with the principles outlined in the Declaration of Helsinki.

\subsection*{2.2 Preparation of cell lysates and western blotting}

Myocardial tissues were thawed on ice and homogenized using 50 mM Tris (pH 7.6), 150 mM NaCl, 1% sodium dodecyl sulfate, 0.1% NP-40, 40 mM phenyl methyl sulfonyl fluoride (PMSF) to generate cell lysates. Protein concentrations in cell lysates were determined using the Bradford assay. Samples containing equivalent quantities of protein were analysed by western blotting using anti-p53 (R&D Systems, USA), anti-MDM2 (Santa Cruz Biotechnology, USA), anti-ubiquitin (Zymed, USA), anti-E1 (Santa Cruz Biotechnology), anti-HAUSP (Santa Cruz Biotechnology), anti-caspases 3, or anti-caspase 9 (R&D Systems), or with anti-poly (ADP ribose) polymerase-1 (PARP-1), anti-CAD or anti-ICAD primary antibodies (all from Santa Cruz Biotechnology), horseradish peroxidase-conjugated secondary antibodies (Dako, Denmark), and chemiluminescent detection. Protein loading was normalized by western blotting using anti-GAPDH antibodies (Santa Cruz Biotechnology). Expression levels of particular proteins were quantified by laser densitometry of specific bands on autoradiographs and standardized to total protein levels in each respective lane. Densitometric analysis was carried out using the QUANT ONE software (Biorad, USA).

\subsection*{2.3 Activity profiling of deubiquitinating enzymes}

Cytosolic lysates were made from tissue samples by homogenization using 50 mM Tris (pH 7.6), 0.2% NP-40, 150 mM NaCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM 4-(2-amino ethyl)benzene sulfonyl fluoride (AEBSF). Synthesis and purification of the thiol-reactive, ubiquitin-derived probe used in this study (HAUbVME) has been described previously.\textsuperscript{29} It is tagged with a hemagglutinin (HA) epitope to facilitate detection. Probe was applied to cytosolic lysates and reactions were incubated at 37°C for 1 h. Probe sequences were detected by western blotting using anti-HA epitope antibodies (1:1000; Roche, Switzerland), and chemiluminescent detection. Protein loading was normalized by western blotting using anti-GAPDH antibodies (Santa Cruz Biotechnology). Expression levels of particular proteins were quantified by laser densitometry of specific bands on autoradiographs and standardized to total protein levels in each respective lane. Densitometric analysis was carried out using the QUANT ONE software (Biorad, USA).
HRP-conjugated secondary antibodies, and chemiluminescent detection. Enzyme-probe complexes in test samples were identified by comparing their migration on polyacrylamide gels with complexes identified previously in cardiac tissues by mass spectrometry.

2.4 Immunocytochemistry

Frozen myocardial sections (5-6 μm) were blocked using 1% bovine serum albumin containing 1% Tween-20. The sections were stained using anti-p53 (BD Systems), anti-MDM2 (Santa Cruz Biotechnology) or anti-CAD (Santa Cruz Biotechnology) antibodies, or with antibodies that specifically recognize cleaved, active forms of caspases-3 or -9 (BD Systems). Isotype-matched monoclonal antibodies raised against irrelevant antigens or pre-immune rabbit sera were used as experimental controls for specific staining. Sections were washed using phosphate-buffered saline before application of biotinylated rabbit anti-mouse or swine anti-rabbit immunoglobulins (Dako). Antibody binding was detected by application of extravidin peroxidase complex, diaminobenzidine tetrahydrochloride (25 mg/mL) and hydrogen peroxide (0.01% w/v). All slides were counterstained in Mayer’s haematoxylin.

2.5 Assay of 20S-proteasome chymotrypsin-like activity

20S-Proteasome chymotrypsin-like activities were measured in cytosolic lysates using a fluorescent Suc-LLVY-AMC substrate (Biomol, USA) as described previously. Cytosolic lysates were made from tissue samples using 50 mM Tris (pH 7.6), 0.2% NP-40, 150 mM NaCl, 0.5 mM EDTA, 0.5 mM AEBSF, and protein concentrations were determined using the Bradford assay. Samples containing 30 μg protein were combined with 28 μM ATP and 18 μM Suc-LLVY-AMC and incubated at 37 °C in a fluorescence microplate reader (Synergy HT, BioTek, USA). Fluorescence (excitation 380 nm, emission 440 nm) was measured at 2 min intervals for up to 160 mins. Values were normalized by measuring fluorescence from parallel reactions carried out in the presence of a proteasome inhibitor (20 μM MG132) to control for proteasome-independent chymotrypsin-like activity. Average rates of fluorescence for each reaction were calculated by linear regression using KC4 software (Synergy HT, BioTek).

2.6 Data analysis

A Kruskal–Wallis test for one-way analysis of variance on ranks followed by the Dunn’s test was used for pairwise comparisons against the control group. A P-value <0.05 was considered statistically significant.

3. Results

3.1 Dilated cardiomyopathy tissues contain increased levels of p53 and minute double minute 2

Western blotting revealed that DCM tissues contained higher levels of p53 compared with non-failing hearts (Figure 1A upper panel). These findings are supported by immunocytochemistry which revealed that p53 was expressed at high levels in endothelial and interstitial cells and to a lesser extent in cardiomyocytes in DCM tissue, but was not expressed in non-failing hearts (Figure 1B, compare panels 1 and 2). We next examined whether MDM2, a key negative regulator of p53 stability, was expressed at altered levels in DCM. Surprisingly, DCM tissues contained higher levels of MDM2 protein in cardiomyocytes compared with non-failing control tissues as revealed by western blotting (Figure 1A centre panel) and immunohistochemistry (Figure 1B, compare panels 3 and 4).

3.2 Dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy

We reasoned that the co-existence of high levels of p53 and MDM2 in DCM may be caused by a reduction in the capacity of the ubiquitin ligase MDM2 to target p53 for ubiquitination and proteasomal processing. We therefore examined whether components of the UPS were altered in DCM. Western blotting revealed that levels of polyubiquitinated proteins were elevated in DCM tissues compared with non-failing hearts (Figure 2A). We also detected elevated levels of E1A and E1B ubiquitin-activating enzymes in DCM compared with non-failing control tissues by western blotting (Figure 2A). In vitro assays using a specific fluorogenic substrate revealed that 20S-proteasome chymotrypsin-like activities were significantly increased in DCM tissues compared with non-failing hearts (Figure 2B). Thus the build up of p53 proteins in DCM cannot be attributed to suppression of either ubiquitination or proteasomal catalytic activities at a ‘global level’.

We hypothesized that p53 may be rescued from ubiquitination and subsequent proteolytic degradation in DCM by deubiquitinating enzymes which are known to cleave ubiquitin from substrate proteins. First, we assessed the activities of multiple deubiquitinating enzymes in cytosolic lysates made from DCM or non-failing control tissues using a thiol-reactive ubiquitin-derived probe. Several deubiquitinating enzymes including USP15, USP14, UCH37, UCH-L3, and UCH-L1 were detected in both non-failing and DCM tissues (Figure 3A). The activities of the two enzymes, USP14 and USP15, appeared to be elevated in DCM tissues compared with non-failing hearts, but these differences did not reach statistical significance (Figure 3A, right panels). In addition, western blotting revealed that expression levels of HAUSP, an enzyme that targets p53 for deubiquitination, are significantly elevated in DCM compared with non-failing control tissues (Figure 3B). These findings are supported by immunocytochemistry which revealed elevated expression of HAUSP in cardiomyocytes of DCM tissues compared with non-failing hearts (Figure 3C, compare panels 1 and 2). Thus, our data indicate that elevated p53 levels in DCM tissues are associated with enhanced expression of HAUSP, a molecule that can rescue ubiquitinated p53 from proteasomal processing.

3.3 Positive and negative regulators of DNA fragmentation are upregulated in dilated cardiomyopathy

To assess the physiological significance of elevated p53 expression in DCM, we examined whether it was associated with activation of caspases. Western blotting of cell lysates revealed that DCM tissues contained significantly higher levels of active forms of caspases-9 and -3 compared with non-failing hearts (Figure 4A). These data are consistent with immunocytochemistry which revealed active forms of caspases-9 and -3 in the nuclei of cardiomyocytes in DCM tissue but did not identify active caspases in non-failing hearts (Figure 4B, compare panels 1, 2 and 3, 4). DCM tissues also contained significantly reduced levels of intact PARP-1 (Figure 4A), which is a known caspase substrate. We next examined whether the expression of CAD, a key effector of DNA fragmentation in apoptosis, and its inhibitor ICAD was altered in DCM. Western blotting
revealed that DCM tissues contained elevated expression levels of CAD (Figure 4A). We also observed that DCM tissues contained reduced levels of a 45 kDa variant of ICAD (ICAD-L) and elevated levels of ICAD-S, an alternatively spliced 35 kDa form which is known to inhibit CAD more effectively than ICAD-L (Figure 4A). Thus, we conclude that DCM is associated with elevated levels of both positive (CAD) and negative (ICAD-S) regulators of DNA fragmentation.

4. Discussion

Our study revealed that human DCM is associated with elevated protein levels of the pro-apoptotic transcription factor p53 and minute double minute 2 (MDM2) are expressed at elevated levels in dilated cardiomyopathy (DCM) tissues. (A) Cell lysates from DCM and non-failing (NF) heart tissues were tested by western blotting using anti-p53 or anti-MDM2 antibodies. Total protein levels were normalized by testing lysates using anti-GAPDH antibodies. Representative blots generated using a subset of samples are shown (left panels). The average quantities of specific proteins were determined for DCM (n = 12) and NF (n = 17) groups by densitometry of autoradiographs (right panels). Mean optical densities are presented with standard deviations (arbitrary units; §P < 0.001; *P < 0.05). (B) DCM or NF tissues were tested by immunocytochemistry using either anti-p53 or anti-MDM2 antibodies or isotype-matched antibodies that recognize an irrelevant protein (control IgG). Representative images are shown with positive cardiomyocytes (arrows) and interstitial cells (arrowheads) identified. BV, blood vessel. Bar indicates 100 μm.
p53. To investigate the underlying mechanism, we examined the expression of MDM2, an E3 ligase that can destabilize p53 by targeting it for ubiquitination and proteasomal degradation.25–27 We observed that MDM2 was expressed at elevated levels in DCM compared with non-failing human hearts, a finding that is consistent with previous observations that MDM2 expression can be elevated by pressure overload in feline31 or murine32 hearts. It is somewhat surprising that elevated MDM2 levels are associated with raised p53 expression given the capacity of MDM2 to reduce p53 stability. We therefore examined whether ubiquitination and proteasomal catalytic activities were altered in DCM. Our observations revealed that polyubiquitinated proteins build up at elevated levels in DCM tissues despite enhanced proteasomal activities. This suggests that the UPS may be overwhelmed in DCM by the production of excessive amounts of polyubiquitinated cellular proteins that exceed the degradative capacity of the proteasome. It follows that polyubiquitinated p53 proteins may be prevented from engaging with proteasomes and therefore be degraded at a diminished rate in DCM, thus elevating p53 expression levels. In addition, we demonstrate that HAUSP, an enzyme that cleaves ubiquitin from modified forms of p53, is expressed at elevated levels in DCM compared with non-failing control tissues. It is conceivable therefore that modified p53 may be rescued from proteasomal processing in DCM by deubiquitination. Thus, we suggest that HAUSP expression and UPS dysregulation may play key roles in elevating p53 expression in end-stage DCM.

The level of polyubiquitinated proteins is governed by a balance between the activities of E1, E2, and E3 enzymes which regulate ubiquitination, the activities of deubiquitinating enzymes which remove ubiquitin from modified proteins and proteasomes which degrade polyubiquitinated proteins. Given that proteasomal catalytic activities are elevated and that the activities of the majority of deubiquitinating enzymes are unchanged in DCM, it is likely that polyubiquitinated proteins accumulate in DCM due to high rates of ubiquitination. This idea is consistent with our observation that DCM is associated with elevated levels of E1 ubiquitin-activating enzymes which carry out the first step in a chain reaction that leads to ubiquitination of cellular proteins and with a previous observation that numerous E3 ubiquitin ligases are expressed at elevated levels in a feline model of pressure overload.31 High polyubiquitination rates could also be triggered by the overproduction of UPS substrates in DCM either as a result of increased metabolic activity or by misfolding of cellular proteins in response to physiological stress.33–35 The UPS regulates numerous fundamental physiological activities in cardiomyocytes including apoptosis,36 hypertrophy,37 contractile function, and signalling, and emerging reports

Figure 2 20S-Proteasome chymotrypsin-like activities and levels of ubiquitinated proteins are elevated in dilated cardiomyopathy (DCM). (A) Western blotting was used to compare the levels of polyubiquitinated proteins, monoubiquitin, E1A, and E1B proteins in total cell lysates from DCM and non-failing (NF) tissues. Representative blots generated using a subset of samples are shown (left panels). The average quantities of specific proteins were determined for DCM \( (n = 12) \) and NF \( (n = 17) \) groups by densitometry of autoradiographs (right panels). Mean optical densities are presented with standard deviations (arbitrary units: \( *p < 0.01 \)). (B) In vitro assays employing a specific fluorogenic substrate were used to measure 20S-proteasome chymotrypsin-like activities in cytosolic lysates made from DCM or NF tissues. Enzymatic activities are presented as mean rates of fluorescence with standard deviations (arbitrary units: \( §p < 0.04 \)).
suggest that its dysregulation may play a role in the pathogenesis of DCM. Indeed, pressure overloading of murine6 or feline31 hearts leads to the accumulation of ubiquitinated proteins before the onset of cardiac dysfunction. Thus, our suggestion that the UPS may be overwhelmed in end-stage human DCM because of the overproduction of aberrant or cytotoxic proteins has important implications for our understanding of DCM pathogenesis.
Our observation that DCM is associated with elevated levels of p53 and with activation of caspases is consistent with previous reports. However, it remains uncertain whether caspase activation is capable of triggering apoptosis in HF or whether the pathway is interrupted. We addressed this issue by examining CAD, a molecule that regulates DNA fragmentation which is an irreversible step in programmed cell death. In healthy cells, CAD interacts with an inhibitory molecule called ICAD that suppresses its catalytic activity. Pro-apoptotic stimuli activates...
caspases which cleave ICAD, thus liberating CAD for DNA fragmentation. Recent studies have revealed that ICAD can be expressed either as a long form (ICAD-L; 45 kDa), or as an alternatively spliced short form (ICAD-S; 35 kDa) and that ICAD-S inhibits CAD more effectively than ICAD-L. We observed that DCM tissues contained elevated levels of CAD but we did not find evidence for caspase-dependent cleavage of ICAD. Indeed, we observed that DCM tissues contained elevated levels of ICAD-S which is a particularly potent form of the inhibitor. Thus, elevated p53 expression and caspase activation in DCM was not associated with activation of CAD, a finding that is consistent with a previous report that DNA fragmentation is rare in DCM. It is conceivable that elevation of ICAD-S represents a mechanism to suppress DNA fragmentation and thereby impair apoptotic processes in HF.

In summary, our data suggests that elevated p53 expression in DCM may arise because of alterations in UPS components that govern its stability. Elevated p53 expression in DCM may arise because of alterations in UPS-dependent cleavage of ICAD. Indeed, we observed that DCM tissues contained elevated levels of ICAD-S which is a particularly potent form of the inhibitor. Thus, elevated p53 expression and caspase activation in DCM was not associated with activation of CAD, a finding that is consistent with a previous report that DNA fragmentation is rare in DCM. It is conceivable that elevation of ICAD-S represents a mechanism to suppress DNA fragmentation and thereby impair apoptotic processes in HF.

Funding
British Heart Foundation, Magdi Yacoub Institute.

Acknowledgement
We thank Joseph Boyle for critical reading of the manuscript.

Conflict of interest: none declared.

References

