Peer-Review Record

Cytosolic DNA sensing protein pathway is activated in human hearts with dilated cardiomyopathy

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Academic Editor: Sherif F. Nagueh

Reviewer 1: Anonymous

Reviewer 2: Anonymous

Round 1

Reviewer 1 Report

Please provide additional information on how the DCM and the control hearts were selected. For the DCM hearts, please explain where the samples were obtained from: were they all from the left ventricle, which wall, and whether they were subendocardial or transmural samples. There is a need to comment on whether the findings are indeed representative of the whole ventricle. The CGAS pathway activates inflammation. It is not clear if there was evidence of inflammation previously in these hearts or by histopathology at the time the hearts were sectioned for this report. The etiology of DCM should be included in the manuscript and whether there are previous data, by biopsy or CMR, to support the presence of inflammation.

Author Response

Please provide additional information on how the DCM and the control hearts were selected. For the DCM hearts, please explain where the samples were obtained from: were they all from the left ventricle, which wall, and whether they were subendocardial or transmural samples. There is a need to comment on whether the findings are indeed representative of the whole ventricle. The CGAS pathway activates inflammation. It is not clear if there was evidence of inflammation previously in these hearts or by histopathology at the time the hearts were sectioned for this report. The etiology of DCM should be included in the manuscript and whether there are previous data, by biopsy or CMR, to support the presence of inflammation.

We thank the Reviewer for the informative and constructive comments. Please kindly note that the information about these samples have been published, as cited in the manuscript

(Chen et al 2019 and Cheedipudi et al 2019). The human heart samples were stored samples that were collected several years ago from patients with DCM undergoing heart transplantations. The samples are transmural and all from the left ventricular tissues. However, unfortunately, we do not know exactly which segment(s) of the left ventricle were excised during the explantation and used in the present studies. Such information was not collected at the time of excision of the samples. This shortcoming has been added to the revised manuscript.

We appreciate the reviewer's point about activation of the pro-inflammatory genes by CGAS, mainly through the pathways that we have examined in this study (NFkB1 and IRF3). As the reviewer would appreciate, the data provides compelling evidence for the activation of the NFkB1 pathway in the human heart samples with DCM. In response to the reviewer's comment, we have analyzed the published RNA-Seq data in these samples for the expression of pro-inflammatory genes and have included a new figure (Figure 3) showing upregulation of over two dozen genes involved in inflammation. The manuscript has been revised accordingly.

Revisions:
Page 4. All samples were from the left ventricular tissues and were transmural, however, the exact segment(s) of the left ventricles that were excised and used in these experiments was unknown.

Page 7. The findings also might be influenced by the presence of regional differences in the myocardial gene expression and cellular composition, as the exact segment(s) of the left ventricles that were excised and used in these experiments were not defined at the time of sample collection. 21, 22 In addition, the samples were stored for several years at -130 degrees, which might affect the integrity of the transcripts and proteins, particularly the phosphorylated forms of the selected proteins.

Page 6. To determine whether increased protein levels of the components of the NFB pathway was associated with the upregulation of expression of genes involved in inflammation, transcript levels of over two dozen genes were analyzed in the RNA-sequencing data. 13, 18 The data show increased transcript levels of 26 genes encoding proteins involved in the inflammatory pathways, including members of the cytokine and tumor necrosis factor family (Figure 3).

Reviewer 2 Report

This is a study that documents evidence for activation of the cytosolic DNA sensing protein pathway in hearts from human patients with dilated cardiomyopathy. The findings are that, similar to those in a mouse model, the DNA damage response and cytosolic DNA-sensing proteins (CDSPs), such as cyclic GMP- AMP synthase (CGAS) pathways are upregulated in DCM patients. The authors claim that this paper is the first to show activation of this pathway in diseased human hearts.

Overall, the paper is very descriptive in nature, which is acknowledged by the authors. Given that this study was performed on human heart samples, additional ‘mechanistic’ studies would be difficult if not impossible.

The paper is a bit difficult to read because it is not formatted into conventional sections. I recommend reorganizing the manuscript into the usual sections like introduction, methods, results, and discussion.

In the methods section, the manuscript lacks critical information about IRB approval. Details and protocol number should be included. Also, please include information about the harvesting procedure, how the samples were stored/frozen (time to freeze clamp), and exactly what areas of the heart were studied.

Moreover, were these studies performed by investigators blinded to the sample identity?

In the results section, a table with patient demographics should be included.

Would it be possible to increase the sample size? Only 2 of the 6 markers are significant, which might raise the question whether the pathway is indeed activated in most samples?

Please provide exact fold change with error for all the results. Please include the statistical tests used in all the analyses.

Did the authors exclude the presence of viral DNA which might activate the pathway?

Please correct small typos.

Author Response

This is a study that documents evidence for activation of the cytosolic DNA sensing protein pathway in hearts from human patients with dilated cardiomyopathy. The findings are that, similar to those in a mouse model, the DNA damage response and cytosolic DNA-sensing proteins (CDSPs), such as cyclic GMP- AMP synthase (CGAS) pathways are upregulated in DCM patients. The authors claim that this paper is the first to show activation of this pathway in diseased human hearts.

Overall, the paper is very descriptive in nature, which is acknowledged by the authors. Given that this study was performed on human heart samples, additional ‘mechanistic’ studies would be difficult if not impossible.

The paper is a bit difficult to read because it is not formatted into conventional sections. I recommend reorganizing the manuscript into the usual sections like introduction, methods, results, and discussion.

Please kindly note that we have followed the format of the Journal for Brief Reports, which recommend the main body of the manuscript to be unstructured.

In the methods section, the manuscript lacks critical information about IRB approval. Details and protocol number should be included. Also, please include information about the harvesting procedure, how the samples were stored/frozen (time to freeze clamp), and exactly what areas of the heart were studied.

Please kindly note that per instructions of the Journal, the IRB approval and the protocol number were included at the end of the manuscript and not within the body of the manuscript.

Please kindly note that the information about these samples have been published (Chen et al 2019 and Cheedipudi et al. 2019). The explanted heart samples were from patients with primary DCM who underwent heart transplantation due to refractory heart failure. The control hearts from donors who died of non-cardiac causes (trauma) and were not used for transplantation. We have added information about harvesting procedure and storage of the samples to the manuscript. Unfortunately, we do not have detailed information about the exact areas of the heart that were examined, as these.

Revisions:
Page 4. All samples were from the left ventricular tissues and were transmural, however, the exact segment(s) of the left ventricles that were excised and used in these experiments was unknown.

Page 7. The findings also might be influenced by the presence of regional differences in the myocardial gene expression and cellular composition, as the exact segment(s) of the left ventricles that were excised and used in these experiments were not defined at the time of sample collection. 21, 22 In addition, the samples were stored for several years at -130 degrees, which might affect the integrity of the transcripts and proteins, particularly the phosphorylated forms of the selected proteins.

Moreover, were these studies performed by investigators blinded to the sample identity?

The investigators were not blinded and were aware of origin of the samples used in the immunoblotting experiments. We note that an equal amount of each heart tissue sample was used in the immunoblotting experiments and proper loading controls are included. In addition, immunoblotting experiments, in contrast to some of the other technique is less amenable to ascertainment bias or other operator -dependent biases.

Revisions:
Page 7: The findings might be subject to data ascertainment and analysis bias, as immunoblotting was performed with the knowledge of the groups. However, to reduce the potential bias, an equal amount of each left ventricular tissue was used in these experiments and the loading controls were included and used for data normalization.

In the results section, a table with patient demographics should be included.

Please kindly note that the demographic data n these cases have been published. We have referenced the published data. (Chen et al 2019 and Cheedipudi et al 2019). Therefore, only a brief information was included. We trust the reviewer would agree that it is best to reference the published data rather than duplicate it.

Would it be possible to increase the sample size? Only 2 of the 6 markers are significant, which might raise the question whether the pathway is indeed activated in most samples?

Originally, we had 5 samples (as in the published data), but we ran out of one sample. Unfortunately, there are no additional tissue sample, and we are not able to collect new samples, which would take years. We have acknowledged the limitation of this study.

Please note that data in Figure 1 and 2 all pertain to the CDSP pathway and that CGAS, TBK1, RELB, P52, and P50 all were upregulated.

Revisions:
Page 7: The study has several limitations, including the small sample size of the cases and controls as well as its descriptive, albeit informative, nature. Thus, confirmation of the findings in larger sample-size studies would be valuable.

Please provide exact fold change with error for all the results. Please include the statistical tests used in all the analyses.

Please kindly note the data has been presented as Box and Whisker plots that contain the individual data points, median values, and the minimum-maximum values. We have clarified this in the figure legend and to avoid redundancy, we have not added the quantitative data to the main text of the manuscript.

We regret missing the statistical methods and greatly appreciate the Reviewer's reminder. We have added the statistical methods used in the revised manuscript as follows:

Revisions:
Page 12. Box and whisker plots are shown and include the individual data points, the median, and the minimum and maximum values. All data followed the Gaussian distribution, as analyzed by the Shapiro-Wilk normality test. The differences between the two groups were compared by unpaired t-test.

Did the authors exclude the presence of viral DNA which might activate the pathway?

We have not tested these samples for the presence of viral DNA. This shortcoming has been added to the revised manuscript.

Revisions:
Finally, the human heart samples were not analyzed for the presence of viral DNA or RNA, reflective of viral infection, which is known to activate the CGAS-STING1 pathway[9, 10, 23].

Please correct small typos.

Thank you. We have corrected the typos.