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Heart diseases

NEW FUNCTIONS OF THE APOPTOTIC GENES IN THE DEVELOPMENT AND STRESS OF THE MYOCARDIUM

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1. Project summary

The functioning of the heart is influenced by a network of genes that acts during development by determining the correct number of cardiomyocytes, their growth and maturation. In adults, the interruption of the supply of oxygen and nutrients induces myocardial death, which can lead to heart failure. Apoptotic genes were investigated as targets to prevent cell death, but it is currently known that genes that regulate apoptosis could be important for proper cardiac development. Previous research suggested that (1) some apoptotic genes such as executioner caspases and nucleases regulate myocardial growth and differentiation, and that (2) the maintenance of mitochondrial integrity of cardiac fibroblasts, which generate the fibrous scar in a heart attack, bases its high survival. Based on these facts, our starting hypotheses were that (1) caspases-3 and 7 and Endog and Tatdn1 nucleases are necessary for the correct division of myocytes during development, and (2) cardiac fibroblasts use autophagy in case of lack of nutrients, such as during ischemia, to remain functional. To answer these hypotheses, we set as objectives the identification of the molecular mechanisms by which caspases influence the division and maturation of myocytes, as well as to find out a possible association of apoptotic genes with cardiac function. We also wanted to identify the mechanisms by which the Endog nuclease controls cell division and growth in the heart, as well as to characterize the role of the Tatdn1 nuclease in the myocardium. On the other hand, we wanted to characterize autophagy in cardiac fibroblasts in ischemia and their relevance for the metabolic activity of these cells.

The biochemical characterization of the mouse heart where we interrupted the genes of the executioner caspases 3 and 7 to the myocardium indicates that there is more inflammation and fewer myocytes, and that these are larger than in normal mice. In addition, we conducted a GWAS genomic study including 11,500 people from genetic databases where we analyzed the possible association between cardiac function and changes in the sequences of 20 apoptotic genes as well as a global agnostic study. This study allowed us to confirm the association of some apoptotic regulators BCL2, CFLIP (cFLAR), FADD, Caspase-8, Caspase-10 and TATDN1 with changes in cardiac function markers but also allowed us to identify other genes potentially relevant for cardiac function and unexpectedly, a link between genes associated with cardiac function (including some apoptotic genes) and the development of Alzheimer's disease. This group of data confirm the influence of apoptotic genes on heart development and

cardiac function and also suggest a link between cardiac function and Alzheimer's disease.

Molecular, biochemical and cellular analysis of the models of mice deficient in the Endog and Tatdn1 nucleases as well as studies in cardiomyocytes and other cells in culture showed that the lack of Endog both in vivo and in vitro generates oxidative stress that induces changes in the cellular signaling that results in myocyte hypertrophy and slowing cell proliferation of both myocardium and other cell types including tumor cells. Likewise, we found that the lack of the Tatdn1 gene in mice generates an increase in ventricular diameter and changes in the expression of many genes suggesting that it has an important function in both intracellular and extracellular transport of proteins. Together, these data confirm an important role of Endog and Tatdn1 in cardiac biology influencing the size and number of myocytes, but also the role of Endog in other cells including some tumors, involving the control of oxygen free radicals (ROS) and protein transport.

The biochemical and functional characterization of cardiac fibroblasts under normal conditions, of ischemia and endoplasmic reticulum stress has involved the analysis of cellular respiration, the study of the state of cellular oxidation, the quantification of survival and proliferation capacity, the study of mitochondrial organization and the characterization of gene and protein expression in these cells, comparing them with lung and skin fibroblasts in various situations of cellular stress. The results ruled out an important role of autophagy due to the survival of cardiac fibroblasts during ischemia, but they showed that cardiac fibroblasts have biological characteristics that make them especially capable of surviving low oxygen pressures and conditions that alter the distribution of intracellular calcium, which involve a higher rate of oxygen consumption, more respiratory chains due to mitochondria and high levels of ROS but better control of these in stressful situations, among others. The STAT3 signaling pathway is particularly activated and regulates both the survival and the expression of protective proteins in these fibroblasts. This data block provides new data on the biology of cardiac fibroblasts and their high survival capacity.

In summary, the results obtained confirm the involvement of apoptotic genes in the development and function of the myocardium and demonstrate that some of these genes are regulating myocyte growth and proliferation processes involving the control

of oxygen free radical levels, the Inflammation pathways and control in protein transport. Likewise, we provide new data on cardiac fibroblasts that allow us to better understand how these important cells work in response processes to stress situations in the heart. Finally, the data obtained suggest that some of the genes important for cardiac function, including some apoptotic genes, are also relevant and, therefore, possible targets, in Alzheimer's disease and some types of cancer.

2. Results obtained

Task 1. Functions of apoptotic executor caspases 3 and 7, and nucleases Endog and Tatdn1 in the development of the heart

. There is an increase in myocardial pro-inflammatory cytokines due to the lack of caspase-3 and 7 executioner caspases specifically in this mouse tissue.

. The lack of Endog in rodent cardiomyocytes induces an increase in the abundance of oxygen free radicals (ROS) and their inhibition with N-acetyl-cysteine is able to stop the hypertrophy derived from the lack of this gene.

. The lack of Endog in rodent and human somatic cells in division retards its proliferation that can be recovered by the addition of ROS detoxifiers.

The lack of Endog is able to slow down in vitro and in vivo the growth of deficient tumors in the PTEN tumor suppressor in mouse models and in human tumor cell lines.

. Endog regulates the Akt / GSK3 / beta-catenin cell signaling pathway through the control of ROS levels with an impact on cell growth and division.

. Our data suggest that although Tatdn1 is identified as a nuclease from experiments using overexpression models, it is not involved in DNA cutting at least in the myocardium and brain since: 1) it is found in the cytoplasm and does not translocate to the nucleus or mitochondria in any stress situation studied, 2) cell extracts enriched in this protein do not show nuclease activity in vitro, 3) the pattern of altered genes in the myocardium (and in the brain) of mice Tatdn1 deficient indicates a role of this gene in protein transport and not in the biology of nucleic acids.

. The lack of Tatdn1 in vivo in mice induces an increase in the diameter of the left ventricle in both systole and diastole, suggesting a role of this gene in cardiac hypertrophy and function.

Task 2. Cardiac fibroblasts: autophagy, its role in stress function and the role of Bcl2.

. Autophagy is important for the survival of fibroblasts of any origin under normal conditions, since their inhibition by genetic methods (silencing of the Atg7 or Bnip3 genes) or pharmacological (3-methyl adenine or chloroquine) induces cell death. Despite this, autophagy does not seem to influence the survival of cardiac fibroblasts during ischemia. Nor does the lack of the Bcl2 gene seem to influence the autophagic flow in these cells.

. Rat cardiac fibroblasts show greater oxygen consumption, more abundance of respiratory chains by mitochondria and a somewhat more fragmented mitochondrial network under normal conditions than other types of fibroblasts.

. Cardiac fibroblasts have more abundance of ROS and ROS detoxifying enzymes such as superoxide dismutase-2 (SOD2) and catalase than other types of fibroblasts under normal conditions and regulate these better in ischemia.

. Cardiac fibroblasts have a particularly high activation status of the STAT3 signaling pathway that influences their ability to survive under conditions of cellular stress and controls the expression of proteins such as ROS and Bcl2 detoxifying enzymes, which protects mitochondria under conditions of ischemia.

Task 3. Genome-wide association studies to identify new loci that were used to study the potential association of cell signaling and apoptosis genes with phenotypes related to the cardiac function of the human heart.

. Of the 20 genes with apoptosis regulatory functions that were analyzed in 11,500 human genomes, we identified a statistically significant association of some polymorphisms with some feature related to cardiac function by the BCL2, CFLIP (cFLAR), FADD, Caspase-8, Caspase-10 genes and TATDN1.

. In addition, the non-directed genomic study (agnostic) identified new polymorphisms in other genes that are associated with cardiac function that can be consulted in the databases https://data.mendeley.com/, https: // doi. org / 10.17632 / 22jdjghnsp.1.

. Totally unexpectedly, the comparison of polymorphisms associated with cardiac function and with the development of Alzheimer's disease allowed us to identify a group of genes that are associated with the two traits, including some apoptotic genes, which suggests a potential link between cardiovascular function and Alzheimer's disease that we intend to continue studying.

3. Relevance with possible future implications

The project entitled "New functions of apoptotic genes in the development and stress of myocardium" was designed with the primary objective of confirming or ruling out the involvement of several apoptotic genes in the biology and behavior of myocardium against harmful stimuli, to identify the intracellular mechanisms involved and to characterize the mechanisms that confer cardiac fibroblasts survival advantage to stress situations such as ischemia. The project, therefore, was characterized by a preclinical basic research profile that would serve as a basis for improving the understanding of the biochemical and molecular fundamentals that govern the function of the heart and its response to stress. The purpose was to find possible useful targets in the diagnosis or treatment of both cardiac function abnormalities and to improve the cardiac condition in pathological situations.

The data generated by our work show that there are indeed genes that had been discovered and studied in the field of apoptosis cell death that in fact have an important role in the development and biology of the myocardium, that these functions are not related with cell death and that they mainly influence the number and size of cardiomyocytes and are related to cardiac function. In addition, we show that cardiac fibroblasts are evolutionarily prepared to survive low oxygen pressures and have a series of molecular mechanisms that make them resistant to situations of cellular stress. Therefore, the results derived from the project propose new molecular targets that would have to allow a better understanding of the biological foundations underlying cardiac hypertrophy, cardiac remodeling processes after a heart attack, their possible treatment and the genetic foundations that determine the function of the heart.

In addition, and unexpectedly, our data establish a genetic link between heart function and Alzheimer's disease and identify Endog as a new gene involved in tumor progression. Therefore, these data can be used to design new diagnostic and therapeutic tools in the field of heart disease, neurodegenerative diseases and some types of cancer.

4. Bibliography

Congress communications

Paralog mitochondrial nucleases ExoG and EndoG have similar roles in cell proliferation but divergent distribution and different impact on apoptosis. Gisel Barés, Natividad Blasco, Aida Beà, Cristina Girón, Marta Llovera, Daniel Sanchis. P19r-51 XL Congreso de la Sociedad Española de Bioquímica y Biología Molecular. Barcelona, October 2017.

Deciphering the molecular signaling that confers high resistance to ischemia in cardiac fibroblasts. Aida Beà, Natividad Blasco, Gisel Barés, Marta Martínez, Miquel Vila, Cristina Girón, Joan Comella, Marta Llovera, Daniel Sanchis. P19r-47. XL Congreso de la Sociedad Española de Bioquímica y Biología Molecular. Barcelona, October 2017.

EndoG regulates cell cycle progression through a ROS dependent mechanism. Gisel Barés, Natividad Blasco, Aida Beà, Cristina Girón, Marta Llovera, Daniel Sanchis. 42è Congreso de la Sociedad Española de Bioquímica y Biología Molecular. Madrid, July 2019.

Cardiac fibroblasts have high basal ROS abundance and electron transport chain activity compared to fibroblasts of other tissues. Beà A, Barés G, Martínez M, Vila M, Cámara Y, Girón C, Comella JX, Martí R, Llovera M, Sanchis D. 42è Congreso de la Sociedad Española de Bioquímica y Biología Molecular. Madrid, July 2019.

Publications

Cardiomyocyte hypertrophy induced by Endonuclease G deficiency requires reactive oxygen radicals accumulation and is inhibitable by the micropeptide humanin. Natividad Blasco, Yolanda Cámara, Estefanía Núñez, Aida Beà, Gisel Barés, Carles Forné, Marisol Ruíz-Meana, Cristina Girón, Ignasi Barba, Elena García-Arumí, David García-Dorado, Jesús Vázquez, Ramon Martí, Marta Llovera, Daniel Sanchis*. Redox Biology. 2018 Jun;16:146-156. doi: 10.1016/j.redox.2018.02.021. * corresponding author.

Genome Wide Meta-Analysis identifies common genetic signatures shared by heart function and Alzheimer's disease. M.E. Sáez, A. González-Pérez, B. Hernández-

Olasagarre, A. Beà, S. Moreno-Grau, I. de Rojas, G. Monté-Rubio, A. Orellana, S. Valero, J. X. Comella, D. Sanchis*, A. Ruiz*. Scientific Reports. 2019 Nov 13;9(1):16665. doi: 10.1038/s41598-019-52724-2.* co-corresponding author.

In preparation

EndoG contributes to the regulation of cell proliferation through the control of reactive oxygen species. Natividad Blasco[1], Aida Beà[1], Gisel Barés, Cristina Girón, Raúl Navarides, López-Lluch G, Xavier Dolcet, Marta Llovera, Daniel Sanchis*. In preparation. 1 these authors contributed equally, *corresponding author.

STAT3 constitutive activation sustains a set of biological functions conferring survival advantage to heart fibroblasts. Aida Beà, Cristina Girón, Marta Llovera*, Daniel Sanchis*. In preparation. *co-corresponding authors.

Databases

https://data.mendeley.com/, https://doi.org/10.17632/22jdjghnsp.1

Doctoral Theses

Natividad Blasco Angulo (member of the project team).
Director: Daniel Sanchis
EndoG influye en el crecimiento y proliferación cel·lular con impacto en el desarrollo de Tejidos y en la progresión tumoral.
Date and mark: 22/09/2017 Excellent Cum Laude.

Gisel Barés Junqué (member of the project team).
Director: Daniel Sanchis y Marta Llovera
Estudi de les funcions no apoptòtiques de les nucleases ExoG i TatD.
In preparation. Expected defense date June 2020.

Aida Beà Tàrrega (hired through the Marató de TV3 project).
Director: Daniel Sanchis y Marta Llovera
Identificació dels fonaments moleculars que confereixen elevada capacitat de supervivència als fibroblasts cardíacs.
In preparation. Expected defense date June 2020.