Conclusion

Chicken hypoxic hearts have increased heart mass/fetal mass ratio. Some of the identified genes are linked to cell division and others have no known function. They all merit further study for potential involvement in cardiac remodelling. With the little information available and although the qPCR did not confirm the microarray results, links between those genes and cell division have been made. This is considered to be a first step, more work should be put on them especially on the similar to ENS-1 gene which is of some importance since it is expressed in undifferentiated embryonic stem cells but is down regulated in induced embryonic stem cell.

Acknowledgement

My warmest gratitude is for my supervisor Jordi Altimiras for all the help, support, guidance and assistance he provided me the whole time it took to realize this study. My gratitude goes also the other member of his lab for the advices and help provided.

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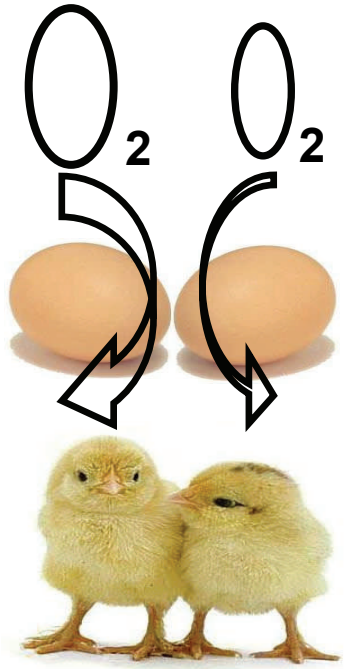
Differential gene expression in the heart of hypoxic chicken fetuses



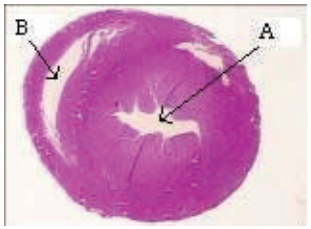
Master's Program in

Molecular Genetics and Physiology

2009

Background

Growth of the heart during the embryonic and fetal periods happens through proliferation of mononucleated cardiac myocytes in a process called hyperplasia. Early in the postnatal period, the cardiac myocytes loose their ability to proliferate and become differentiated as karyogenesis (formation of the nuclei of a cell) is happening in the absence of cytokinesis (cytoplasm division following nucleus division). This process, called cardiac hypertrophy, is specific to organisms. In humans, 90 % of the cardiac myocytes are binucleated in later gestation and up to 97 % are binucleated within seven weeks after birth. In chickens, however, all cardiac myocytes are mononucleated at day one after hatching. The terminal differentiation of cardiomyocytes is first seen after hatching, and 18% of the cardiomyocytes are binucleated at day 15.



Heart section showing (A) left and (B) right ventricle

Aim of the study

Evidence has shown that hypoxic heart have a greater heart mass/fetal mass ratio. But it is still unclear if it is hyperplasia or cardiac hypertrophy that is happening. Furthermore the genes that might be involved in the process have not yet been identified. In the present study, the cardiac transcriptome was analyzed to identify differentially expressed genes related to hypoxia using microarray techniques and quantitativ PCR (qPCR) as major tools.

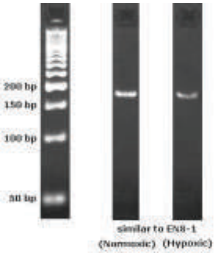
Material and methods

Eggs were incubated and sampled after 15 and 19 days in two different environments: normoxic (21% oxygen) and hypoxic (14% oxygen). The affymetrix chip was used to detect the differentially expressed mRNA. Normalized microarray results were then analyzed to isolate differentially expressed probes. Afterwards total RNA was also isolated from another set of fetuses incubated in the same conditions as the microarray group and used to perform a qPCR in order to confirm the microarray results.

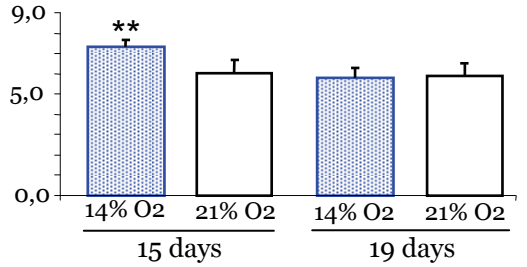
Rotor-gene 6000 used for the realtime PCR

Results

In the four groups (15H, 15N, 19H, 19N), some probes were differentially expressed. From the eggs incubated up to 15 days, the microarray revealed 5 probes that were differentially expressed according to the set criteria (pValue >0.01 and absolute fold change FC>2) in the two programs (PLIER & RMA) used to normalise to data. From the eggs incubated up to 19 days, 8 probes were differentially expressed in both programs. No further tests were performed on the 19 days fetuses since there was no statistical significance in that group after incubation. apo-A1, p22, similar to ENS-1 and β2 adrenergic receptor were further tested in qPCR. The qPCR did not confirm them.



Similar to ENS-1 expression on agarose gel



Ratio (heart weight/fetuse weight)