4-16-2018

Manipulating Fibroblast Environment to Study Specific Gene Expression

Elizabeth Mosqueda
Boise State University

Jericho Schroeder
Boise State University

Dana Small
Boise State University

Caitlyn Dodge
Boise State University

Stephanie Frahs
Boise State University

See next page for additional authors

Manipulating Fibroblast Environment to Study Specific Gene Expression

Abstract

We investigated a model system for cardiac fibrosis. Cardiac fibrosis is the thickening of the heart wall due to the inappropriate proliferation of cardiac fibroblasts and excess deposition of extracellular matrix in the cardiac muscle.

To understand how the cells, respond to stress, we analyzed changes in gene expression. Our research imitated the stress conditions that the heart cells experience. We chose to analyze genes that have not previously been characterized under uniaxial, biaxial and stress-free environments to look at how gene expression varies under different conditions. We normalized all data to a validated housekeeping genes.

This research will help people with various heart problems in repairing damaged tissue. We expect to increase the understanding of the cause of cardiac fibrosis and contribute to a solution. Our conclusions will compare gene expression during healthy conditions to damage repair conditions.

Authors

Elizabeth Mosqueda, Jericho Schroeder, Dana Small, Caitlyn Dodge, Stephanie Frahs, and Julia Oxford

Manipulating Fibroblast Environment to Study Specific Gene Expression

Elizabeth Mosqueda, Jericho Schroeder, Dana Small and Caitlyn Dodge, Stephanie Frahs, Julia Oxford

College of Innovation and Design, College of Arts and Sciences, College of Health Sciences Department of Biological Sciences

Abstract

We investigated a model system for cardiac fibrosis. Cardiac fibrosis is the thickening of the heart wall due to the inappropriate proliferation of cardiac fibroblasts and excess deposition of extracellular matrix in the cardiac muscle.
To understand how the cells, respond to stress, we analyzed changes in gene expression. Our research imitated the stress conditions that the heart cells experience. We chose to analyze genes that have not previously been characterized under uniaxial, biaxial and stress-free environments to look at how gene expression varies under different conditions. We normalized all data to a validated housekeeping genes.
This research will help people with various heart problems in repairing damaged tissue.
We expect to increase the understanding of the cause of cardiac fibrosis and contribute to a solution. Our conclusions will compare gene expression during healthy conditions to damage repair conditions.

Introduction

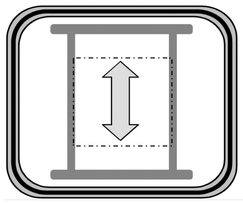
Genes: CD44, CDH2, ECM1, EMILIN1, FN1, ITGA2, ITGA3, ITGA5, ITGAE, ITGAL, ITGAV, ITGB3, NCAM1,were observed under stress free conditions on tissue culture plastic, uniaxial stress and biaxial stress to analyze gene expression a 1 week, 2 week and 3 week period. Each conditions had three specimens. By observing which genes were expressed we can compare the expression in different conditions. At times cells responded to the different stresses by decreasing or increasing the level of the gene expressed. We are trying to imitate more accurate physiological relevance of fibroblasts.

Materials and methods

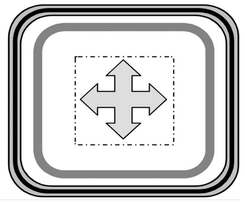
Materials used where C2C12 cells, which are mouse thigh muscle cells. Genes were watched for one, two and three weeks under different conditions: stress free on tissue culture plastic, uniaxial stress and biaxial stress.

Data of expressed genes was analyzed through Microsoft Excel.

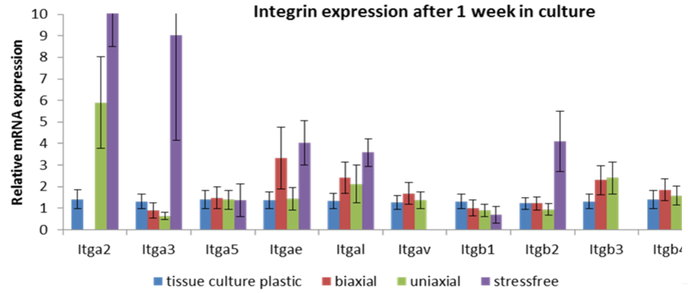
Uniaxial Stretch

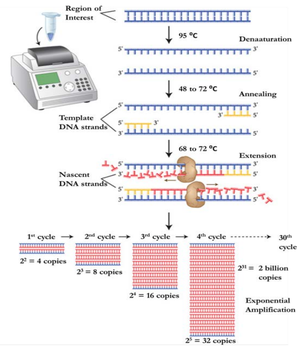


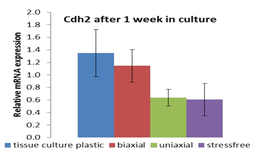
Biaxial Stretch

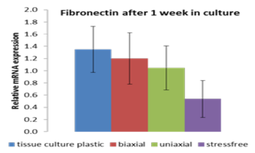


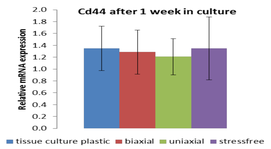
Results

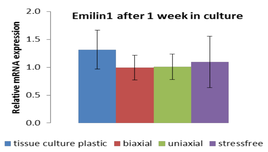


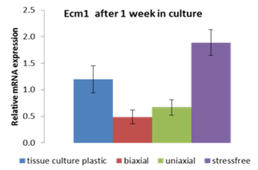


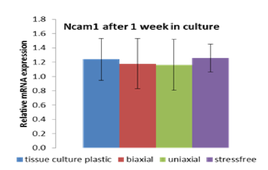




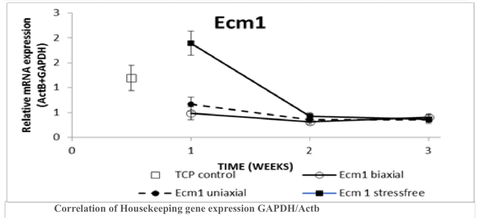


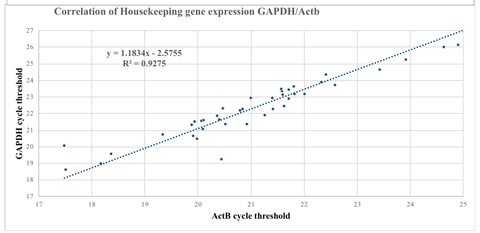






ActB	0	5.957	0.667	5.35	2.725
B2m	5.957	0	6.534	5.821	4.977
GAPDH	0.667	6.534	0	8.188	4.525
GusB	5.35	5.821	8.188	0	0.766
Hsp90ab1	2.725	4.977	4.525	0.766	0
	ActB	B2m	GAPDH	GusB	Hsp90ab1





Conclusions

From these results we can better understand how different genes are expressed under different stress conditions as a way to better identify what is happening in certain situations. We can see how a gene is expressed and try to pinpoint the way that gene is being manipulated by comparing it to the genes found from this experiment. We can also see how observing a gene on tissue culture plastic, which is traditionally how people look at cells, varies from how genes actually behave in cells when they are in ones body. With this data we can also conclude that with studying mutations in genes you can compare the level of expression to see how they correlate to the environment they were placed in.

Further information

FN1 – cell adhesion and migration
ITGA2 – cell and platelet adhesion
ITGA3 – cell surface adhesion molecules
ITGAE – adhesion
ITGAL – intercellular adhesion
ITGA5 – surface adhesion and signaling
ITGB 3 – cell adhesion and surface mediated signaling
CDH2 – development of nervous system, formation of cartilage and bone
EMILIN1 – development of elastic tissues
CD44 – cell-cell interactions, cell adhesion and migration
ITGAV – embryo implantations
NCAM1 – maintains skin integrity and homeostasis
ECM1 – maintains skin integrity and homeostasis

Literature cited

European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics. “Integrin Alpha-V.” *Itgav - Integrin Alpha-V Precursor - Mus Musculus (Mouse) - Itgav Gene & Protein*, 28 Feb. 2018, www.uniprot.org/uniprot/P43406.

Cardiac Fibroblast

Colby A. Souders, Stephanie L.K. Bowers and Troy A. Baudino
Circulation Research. 2009;105:1164-1176, originally published December 3, 2009
https://doi.org/10.1161/CIRCRESAHA.109.209809

Acknowledgments

Authors acknowledge support by Institutional Development Award (IDeA) Program from the National Institute of General Medical Sciences of the National Institutes of Health under Grants #P20GM103408 and P20GM109095. We also acknowledge support from The Biomolecular Research Center. Special thanks to Donald Plumlee, Jimmy Browning, Kenneth Cornell and Trevor Lujan.