Review of Upregulated Gene Products found in the Myocardial Extracellular Matrix post-Myocardial Infarction and Subsequential Enrichment Analysis

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Abstract

The extracellular matrix (ECM) of the heart is not a static scaffold. It is a dynamic structure that responds to stresses and has a constant flow of information through various signaling pathways. After injury, such as cardiomyocyte necrosis due to myocardial infarction (MI), the ECM rapidly changes in attempt to allow the heart to be repaired and continue to function. The upregulation of various gene products help carry out different repair functions, and the outcome of the remodeling is largely dependent on the proper interaction between these proteins and signaling molecules that aggregate in the infarcted area. In order to further analyze and find novel mechanisms in this repair process, it is important to first generate a list gene products that are known to be upregulated in response to MI.

1. Background

The myocardium is the muscular tissue of the heart, and it is composed mainly of cardiomyocytes and fibroblasts which create an extracellular matrix that surrounds these two cell types. The constant cycles of contraction and relaxation of the heart that occur throughout a lifetime along with stresses from significant pressure changes (from 10 mm Hg to 110 mm Hg) require resilient strength and elasticity from the myocardium[2]. Cardiomyocytes are the muscle cells of the heart which form sarcomeres that allow the heart to contract. Collagen in the extracelluar matrix (ECM) provides most of the tensile strength for the myocardium and mostly consists of types I,II,III,IV,V,VI, and XI[2][3]. Collagen proteins are triple helices in structure and are mostly composed of glycine, proline, and hydroxypro-

line. Elastin fibers are the main contributor toward the recoiling capability of the myocardium that follows a contraction, and they are made up of elastin proteins, which are rich in hydrophobic amino acids, crosslinked with glycoproteins[2].

Heart disease is the leading cause of death in both men and women in the United States, the most common cause being from a myocardial infarction (heart attack). A myocardial infarction occurs when there is a blockage in the coronary arteries, causing the heart muscle to lose blood supply, which results in massive cardiomyocyte death. Following cardiomyoctye death, inflammation occurs which causes ECM degredation, and it is replaced by granulation tissue and an acellular collagen-rich scar[2]. Inflammation is stimulated by the TLR4 and NF-κB pathways that secrete various chemokines and cytokines[3]. These signaling chemicals recruit platelets, neutrophils, and mononuclear cells. Remodelling enzymes break down the ECM, then macrophages engulf necrotic cardiomyocytes as well as secrete transforming growth factor β1 which activates fibroblasts thus inducing new ECM synthesis[3]. The balances of ECM degredation, the formation of new functional tissue, and the formation of scar tissue are what determine the success of the repair process. The ECM provides the structural media, signaling pathways, transport pathways, and anchoring points to carry out the healing process and thus the activity in the ECM is indicative of the outcome[3].

2. Methods

The general topic of "ECM" provided some difficulty in getting an appropriate scope for an aggregated gene product list. Even "Cardiovascular ECM" was still quite broad. A lot of time was spent exploring these large gene product lists. Eventually, it was determined to not be feasible. Instead, current research interests in myocardial infarction led to looking into ECM remodeling post-MI as the scope for the gene product list. This proved to seem much more manageable while still being extensive and valuable. Combinations of keywords such as "Extracellular Matrix", "ECM", "Myocardial Infarction", "MI", and "repair proteins" were used to query the Google Scholar search engine as well as FAU's SearchWiSE search engine. Despite FAU's engine being more robust, Google Scholar's search turned up more relevant results more quickly. It was observed that Google Scholar tends to push highly cited articles toward the top of the results list. This is convenient since the most cited articles inherently usually have the most useful

information. Another resource was kindly provided by the course instructor. Protein functions were largely determined directly from journal articles, as most proteins are not specific to the myocardial ECM, protein databases would provide information on general functions of the proteins that were much less relevant.

Papers exist that have utilized microarray analysis to determine differential gene expression post-MI, however results from these papers were omitted as their analysis resulted in many hundreds of differentially expressed genes, and these papers generally already performed enrichment analysis. This paper differs in that it is based on a manually curated list of experimentally reproduced and accepted upregulated gene products who's general function has been determined. Gene ontology enrichment analysis was performed using the GOrilla web interface based on *Mus musculus*.

3. Results

Table 1: Upregulated Gene Products post-MI [1][2][3][4]

Gene	Protein	Function
BGN	biglycan	modulates fibril diameter
COL1A1	collagen I	ECM component
COL3A1	collagen III	ECM component
COL4A1	collagen IV	ECM component
COL5A1	collagen V	ECM component
COL6A1	collagen VI	ECM component
CTGF, CCN2	connective tissue growth factor	promotes fibrosis
DCN	decorin	angiogenesis antagonist
FN1	fibronectin	crosslinks ECM components, guides cell migration
HAS2	hyaluronan	promotes inflammation
HSPG2	perlecan	regulates growth factor signaling
LAMA1	laminin	cell anchor binding site
LGALS3	galectin-3	cell-adhesion, angiogensis, matrix assembly
LOX	lysil oxidase	collagen cross-linking
MMP1	matrix metalloproteinase 1	ECM degradation

Table 1: Upregulated Gene Products post-MI [1][2][3][4]

Gene	Protein	Function
MMP2	matrix metalloproteinase 2	ECM degradation
MMP7	matrix metalloproteinase 1	ECM degradation
MMP8	matrix metalloproteinase 1	ECM degradation
MMP9	matrix metalloproteinase 9	ECM degradation
MMP11	matrix metalloproteinase 1	ECM degradation
MMP12	matrix metalloproteinase 1	ECM degradation
OGN	osteoglycin	regulates maturation of collagen fibers
POSTN	periostin	regulates matrix assembly, helps activate fibroblasts
SDC1	syndecan-1	protects against dilatation
SDC4	syndecan-4	protects against cardiac rupture
SPARC	secreted protein acidic and rich in cysteine	regulates matrix assembly
SPARCL1	hevin	regulates matrix assembly
SPP1	osteopontin	mediates cytokine production, regulates apoptosis
THBS2	thrombospondin-2	prevents cardiac rupture
THBS1	thrombospondin-1	activates growth factor signaling, represses inflammatory signaling
TIMP1	tissue inhibitor matrix metalloproteinase 1	collagenase inhibitor
TIMP2	tissue inhibitor matrix metalloproteinase 2	collagenase inhibitor
TNC	tenascin-C	promotes inflammation, myofibroblast recruitment
VCAN	versican	guides cell migration

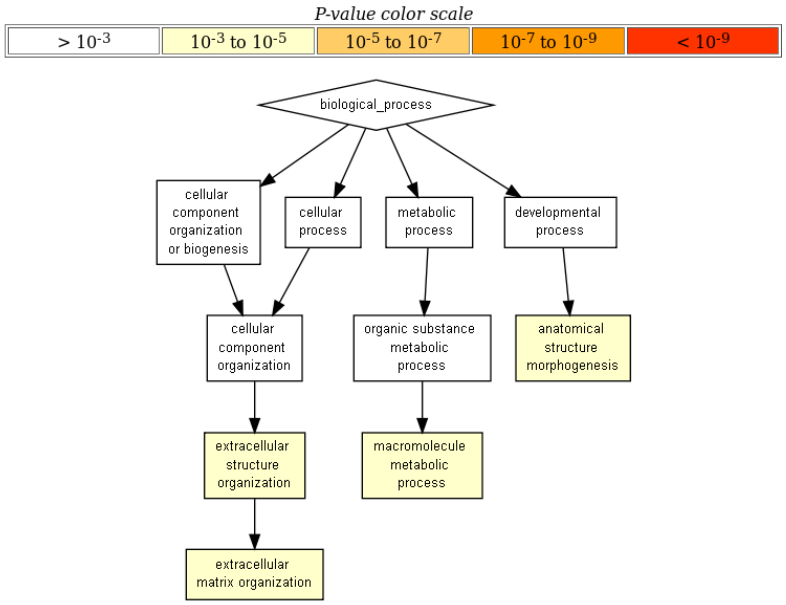

Figure 1: GOrilla Process Enrichment Output

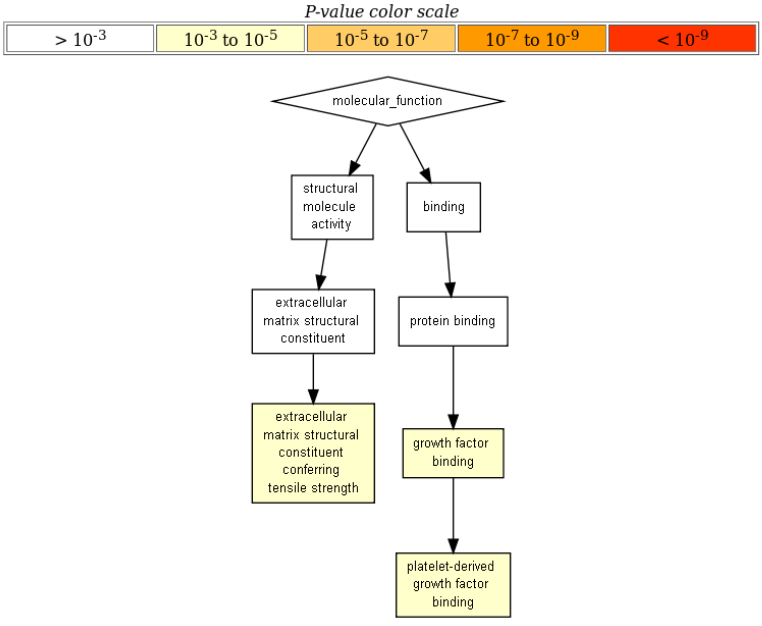

Figure 2: GOrilla Function Enrichment Output

4. Discussion

The functions of many proteins are not fully understood. There role may be different depending on what type of tissue they are in, and they can even have different roles in the same tissue because of a dependence on environmental conditions. The function of many proteins is implied through the effect of their absence in studies using knockout mice. SPARCL1 knockout mice, for example, may show irregular ECM formation post-MI as compared to control. Therefore, SPARCL1 and the protein hevin are assumed to have a role in regulating ECM assembly.

This list is not meant to be exhaustive, it is only a list of known proteins that have a role in post-MI repair as a result of in-vivo experimental data. Attempts at text-mining did not offer any unique information that was not already available from review articles that have been published and referenced in this paper. This manually curated list can be expanded using automated curation methods that could be investigated further and incorporated into the list.

The enrichment analysis results confirmed what was expected. High p-values were allowed (log p-value 3¡ -3) due to the small number of genes being interrogated (35). Metabolic processes occur during the breakdown and subsequent rebuilding of the extracellular matrix. Extracellular matrix organization processes dominate the rebuilding process. Functions of gene products include ECM structures that confer tensile strength and gene products that bind and interact with growth factors for the rebuilding process. It was interesting to see that there were no processes or functions related to inflammation pathways. This indicates that the gene list may be missing some important entries.

5. Conclusion

The myocardial ECM is a dynamic system that allows the continuing function of the heart throughout the stressful conditions it encounters. Remodeling of the ECM post-MI is an important focus area in research as heart disease is the leading cause of death in both men and women in the United States. By aggregating current knowledge of upregulated gene products in response to MI, other pathways and important molecules can be investigated to understand more about the repair mechanisms involved. Gene ontology enrichment analysis helps to confirm these pathways and observe their lineage, as well as critique the validity and completeness of current knowledge.

References

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