Expression Profiling of the Compensatory Response to Myocardial Infarct

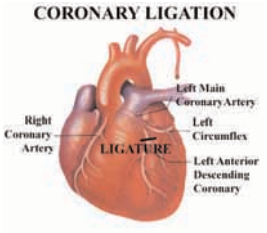
LaFramboise WA[1], Bombach KL[1], GeorgeJD[2], Cullen RF[1], Pogozelski AR[1], Guthrie RD[1],and Magovern JA[1]
Allegheny General Hospital, Cardiothoracic Research, Pittsburgh, PA[1] and Amersham Life Sciences[2], Piscataway, NJ

INTRODUCTION

Myocardial infarction is a multifactorial and polygenic disorder thought to result from genetic predisposition and environmental factors. It is evident that survivors of initial myocardial insult utilize a strategy whereby they compensate for loss of function in the ischemic region by recruiting tissue adjacent to the infarct zone. Oligonucleotide microarrays can probe small amounts of RNA from diverse tissues for widespread gene expression across large segments of the genome without the need for PCR amplification. Therefore, this technology affords the possibility of identifying downstream effectors or interacting pathways involved in the myocardial compensation and remodeling process. The goal of the present study is to characterize gene expression in the infarct and remote zones of male rats recovering from a severe infarct due to left anterior descending coronary artery ligation.

MATERIALS AND METHODS

Coronary Ligation Model



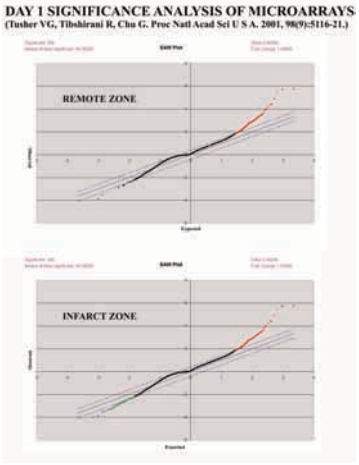
A myocardial infarct was generated by ligation of the left anterior descending coronary artery proximal to the first branch point according to an established procedure. Twenty, ten-week-old (250-300g) male Sprague-Dawley rats were used for the ligation study. Ten animals were sacrificed 24 hours and 28 days post-surgery for tissue harvest to obtain an expression profile of the infarction process allotting for definitive remodeling to take place. Ten animals underwent a sham operation where the artery was isolated but not ligated in order to serve as experimental controls upon sacrifice 28 days later. At sacrifice, myocardial tissue from the left ventricle was immediately dissected in approximately 2-100mg sections and flash frozen in liquid nitrogen for subsequent RNA purification. The two dissected regions were derived from the infarct zone (cardiac apex region) and the tissue adjacent to the infarct region (remote region). Confirmation of the presence and degree of infarction was performed by visual inspection during post-mortem dissection.

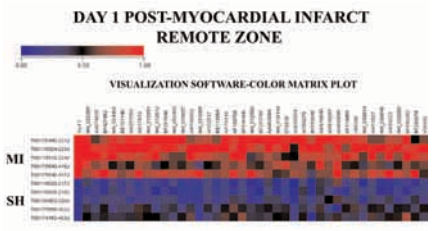
RNA Purification

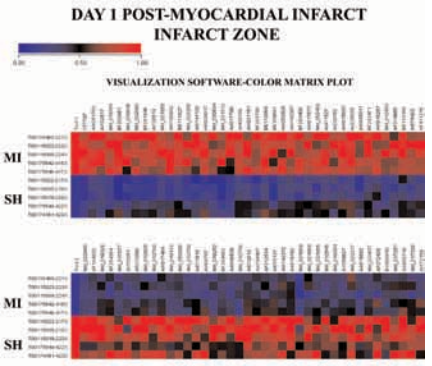
RNA purification was performed using TRIzol reagent (Life Technologies, Gaithersburg,MD) with yields of 100-200 ug of total RNA/100 mg of heart tissue. Total RNA was analyzed to determine that purity met the standards for biochip analysis phase i.e., O.D. 260/280 > 1.8, DNA contamination <5%, and minimal degradation as determined by Agilent Bioanalyzer 2100 electrophoretogram.

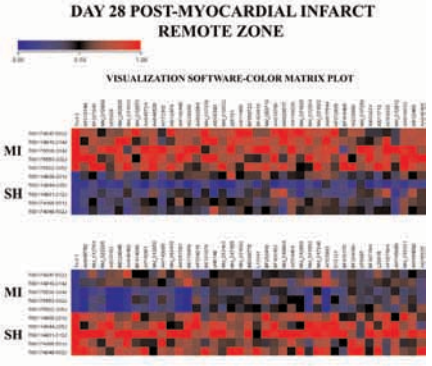
Microarrays and Analysis

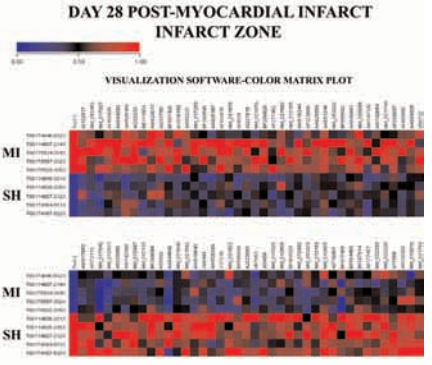
Analysis of mRNA expression levels was performed using RNA oligonucleotide microarrays (Rat 10K Uniset 1, Codelink Microarray System, Amersham Corp., Piscataway, NJ). Preparation of microarrays utilized the Codelink Target Preparation Protocol. Statistical analysis of the gene expression data employed the Significance Analysis of Microarrays (SAM). Normalization by subtraction of threshold and Global Mean Adjustment were the only normalization parameters incorporated into the analysis. SAM was utilized in order to obtain differentially expressed genes.







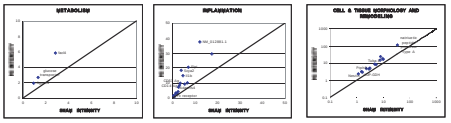




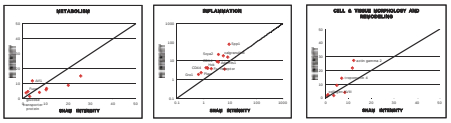
DAY 1 MYOCARDIAL INFARCT RESPONSE

Gene Characterization	INFARCT ZONE Genes Decreased	INFARCT ZONE Genes Increased	REMOTE Genes Increased
Metabolism	6	3	3
Inflammation	1	12	18
Detoxification/Antioxidation	2	2	3
Apoptosis Inducing	0	1	1
Apoptosis Inhibiting	0	2	2
Transcription	2	3	6
Growth Factor Related	2	3	6
Angiogenesis	0	1	1
Protein Synthesis/Degradation	2	4	6
Vesicular Trafficking	0	2	2
Calcium Utilization	1	3	3
Myocardial Contractility	1	0	0
Ion Exchange	6	2	0
Cell &Tissue Morphology/ Remodeling	2	6	15
Signal Transduction/ Proliferation	0	8	7
Miscellaneous	3	4	4
Total Characterized Genes	28	56	77
ESTs	57	140	212
TOTAL GENES	85	196	289

REMOTE ZONE - DAY 1



INFARCT ZONE - DAY 1



DAY 1 vs. 28 DAY ADJACENT REGION

Gene Characterization	DAY 1 Genes Increased	DAY 28 Genes Increased
Metabolism	3	37
Inflammation	18	2
Detoxification/Antioxidation	3	5
Apoptosis Inducing	1	4
Apoptosis Inhibiting	2	1
Transcription	6	17
Growth Factor Related	6	3
Angiogenesis	1	2
Protein Synthesis/Degradation	6	15
Vesicular Trafficking	2	3
Calcium Utilization	3	4
Myocardial Contractility	4	14
Ion Exchange	0	0
Cell &Tissue Morphology/Remodeling	15	21
Signal Transduction/Proliferation	7	16
Total Characterized Genes	77	144
ESTs	212	489
TOTAL GENES	289	683

CONCLUSIONS

- **Compensation begins rapidly among 50% survival group.**
- **Infarct zone demonstrates consistent recovery process.**
- **Metabolic energy derived predominantly from lipid metabolism.**
- **Active detoxification/antioxidative genes activated early.**
- **Immediate and extended transcriptional activation and ongoing signal transduction processes.**
- **Extensive proliferation throughout recovery.**
- **Database of infarct related ESTs established.**