Int.J.Curr.Microbiol.App.Sci (2022) *11*(10): 17-32

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 10 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1110.003

Microaaray Analysis of Differential Gene Expression Profiles of Human Adult Cardiac Myocytes Challenged with Non-Self RNA

Farag A. Bleiblo[1,5*], Souad A. M. Moftah[2], Omar S. Alqabbasi[3], Siham R. Agouri[1], Asma A. Elamari[4], Samira F. M. Bo Hagar[4] and Aseem Kumar[5]

[1]Department of Microbiology, Faculty of Science, University of Benghazi, Libya
[2]Department of Physiology, Faculty of Medicine-Al-Marj Campus, University of Benghazi, Libya
[3]Department of Biology, Faculty of Science-Alwahat Campus, University of Benghazi, Libya
[4]Department of Botany, Faculty of Science, University of Benghazi, Libya
[5]Department of Chemistry and Biochemistry and the Biomolecular Sciences Programme, Laurentian University, Sudbury, ON, Canada

**Corresponding author*

Keywords

Pattern recognition receptors, pathogen-associated molecular patterns

Article Info

Received:
04 September 2022
Accepted:
28 September 2022
Available Online:
10 October 2022

A B S T R A C T

Innate immunity utilizes pattern recognition receptors (PRRs) to sense conserved pathogen-associated molecular patterns (PAMPs) expressed by various pathogenic molecules to activate the initial phase of immune response. Recognition of bacterial RNA by immune sensors induces antigen-specific immunity and secretion of proinflammatory cytokines. Cardiac myocyte dysfunction is clearly identified as underlying the acute heart failure associated with bacterial infections, sepsis, as well as chronic syndrome. Cardiac myocytes express functional PRRs and sense PAMPs directly. Although the immunostimulatory potential and receptor-mediated recognition of nonself RNA are well documented, no comprehensive analysis of differential gene expression in response to naturally occurring bacterial RNA or modified RNA has been reported. Using cDNA Microarrays, we have analyzed the differential gene expression profiles of human adult cardiac myocytes stimulated with bacterial RNA. Our analysis has revealed changes in the cardiac expression profiles of 140 genes. A large proportion of upregulated genes (100) encode proteins involved in regulating the immune responses including, proinflammatory cytokines and chemokines. A significant number of genes involved in stress signalling, homeostasis, and cardiac survival were also induced. We also identified 42 genes to be suppressed. Interestingly, genes implicated in regulation of cardiac cell cycle and transcription were among these repressed genes. Collectively, these Microarray data offer for the first time an insight into human cardiac myocytes response to immunostimulatory RNA such as bacterial RNA.

Introduction

The issue of how to distinguish self from nonself is central to immune system that requires precise regulation of activating and inhibitory signals (Deane and Bolland, 2006; Sioud, 2006). The innate immune system relies on germline encoded PRRs to sense pathogen derived-molecules that are absent in the host (Kawai and Akira, 2007; Erridge, 2008; Akira and Hemmi, 2003). In fact, the discovery of PRRs and the extensive studies have revealed that certain toll like receptors (TLRs) recognize nucleic acids released from infectious organisms and damaged host cell, resulting in modulation of specific signalling pathways to provoke the host response mechanisms (Akira and Hemmi, 2003; Alexopoulou and Kontoyiannis, 2005). This receptor-mediated detection of pathogen-derived nucleic acids is crucial for maintaining the integrity of the genome and for survival. In humans, ten distinct TLRs family members have been reported and the corresponding microbial ligands from different origin have also identified. Although the majority of these receptors sense pathogenic components on the cell surface, TLR3, TLR7, TLR8 and TLR9 which are involved in recognition of nucleic acids, form a distinct subgroup of TLRs based on structural and functional similarities (Bauer *et al.,* 2008; Takeuchi and Akira, 2007). Furthermore, the consideration of innate sensing of self and nonself RNA as a key molecule involved in immune response may extend beyond the TLR field. For example, the RNA-sensing helicases RIG-1 and MDA5, and PKR have been also characterized and identified in anti-viral responses in a TLR-independent manner (Takeuchi and Akira, 2008). Of considerable interest is the recent finding that the naturally occurring bacterial RNA and modified single-stranded RNA (ssRNA) recognized by TLRs can induce antigen-specific immunity, secretion of proinflammatory cytokines and type I interferons (Hamm *et al.,* 2007; Koski *et al.,* 2004). Recent reports have indicated that a substantial number of nucleoside modifications or specific elements are unequally present in either bacterial or mammalians and thus offering a molecular recognition patterns for immune cells to discriminate differences between the immunomodulatory and inert ssRNA (Diebold *et al.,* 2006; Sugiyama *et al.,* 2005). For example, mRNA derived from bacteria without poly A tail, but not from mammalian source, prime for the activation of human myeloid dendritic cells (DCs) (Lan *et al.*, 2007). Unmethylated CpG motifs of ssRNA were immunostimulatory, although 5-C or 2-O methylation of nucleotides in ssRNA abrogates its immunostimulatory activities (Sugiyama *et al.*, 2005).

In addition to being expressed in immune cells, structural cells of cardiovascular system including cardiac myocytes express functional TLRs and can sense bacteria and PAMPs directly (Boyd *et al.*, 2006; Frantz *et al.,* 1999; Frantz *et al.,* 2007; Cartwright *et al.,* 2007). Cardiac myocyte dysfunction is clearly identified as underlying the acute heart failure associated with bacterial infections, septic shock, as well as chronic syndrome. Although a significant progress in the understanding of ssRNA recognition by TLRs and other intracellular receptors have been recently made, no comprehensive analysis of gene expression in response to bacterial RNA has been reported. Therefore, we hypothesized that sensing danger signals of bacterial RNA might modulate the cardiac gene expressions that collectively translate to elicit the end stage of host defence response. Using cDNA Microarrays, we have analyzed the gene expression profiles of human adult cardiac myocytes stimulated with bacterial RNA.

Materials and Methods

Preparation of total bacterial RNA

Pathogenic isolates of *S. aureus* bacteria (capsular serotype 8, nontoxic shock toxin (TSST-1) producer) was used for this study (Paladugu *et al.*, 2004). This strain is known invasive pathogen in immuocompromized humans. We have developed a new reliable method for RNA isolation from *S. aureus*. Basically, this method is a modified hot phenol combined with enzymatic lysis. A 10 ml of

exponentially growing bacteria were harvested by centrifugation at 5000x g in a tube containing 2 ml of ice cold 5% phenol/ethanol stop solution. The cells pellet was resuspended in a fresh lysis buffer of 1000 µl TE pH 8:00, 2 mg/ml lysozyme (Sigma Alderish, St. Loius, USA), 5 µg/ml lysostaphin. Tubes were vortexed and incubated for 30 minutes at room temperature and then 100 µl 20% SDS was added to each tube. After incubation at 64°C for a minute, 40 µl 3M NaOAc was added and mixed. A volume of 850 µl water-saturated phenol was added to each, inverted and incubated for 5 minutes at 62°C. The samples were then chilled on ice and centrifuged at 13000x g for 10 minutes. The aqueous phase was transferred to a fresh 2 ml tube and equal amounts of chloroform were added and centrifuged for 10 minutes at 4°C. The RNA was then precipitated by the addition of 1/10 volume of 1 mM EDTA, 3M NaOAc and 2 volumes 100 % ethanol and incubated for at least 2 h at 80 °C. The pellet was collected, washed with 80% ethanol, and resuspended in100 µl DEPC-treated water. Using this method, we were able to obtain RNA with good yield and purity as evidenced by A260/A280 ratio. To determine the integrity of RNA species, 5 µg from each sample was electrophorsed through 1% agarose gel. Extracted crude RNA was treated enzymatically with RNase-free DNaseI (Roche, Indianapolis, USA) to remove contaminant genomic DNA.

Total RNA isolation and cDNA probe synthesis

Total cellular RNA from human adult cardiac myocytes treated with *S. aureus* RNA and non-treated cells was prepared with extraction using a silica column-based method, Qiagen RNeasy, according to the manufacturer's instructions (Qiagen, Valencia, USA). Prior to the first RPE wash buffer, DNase l incubation mix (1500 Kunits) was added onto RNeasy silica-gel membrane and allowed to sit for 20 minutes to further eliminate any traces of genomic DNA contamination. RNA was quantified by UV spectrometry and electrophorsed on 1% an agarose gel to verify purity and integrity prior to use in Microarray analysis. The Atlas human 1.2 arrays (Clontech, Palo, USA) were used for comparing the gene expression profiles between the controls and *S. aureus* RNA-treated cardiac cells. Each membrane contained the cDNA from 1176 known human genes divided into 6 functional groups. Plasmid and bacteriophage DNA are included as negative controls for hybridization specificity along with nine house keeping cDNA as positive controls. For the first-strand cDNA synthesis, 10 µg total RNA from each sample was mixed with 2 µl CDS primer mix specific for the 1176 genes represented in the atlas human 1.2 array (BD Biosciences Clontech), incubated at 70 C for 3 minutes followed by 2 minutes at 50C. Master mix containing 4 µl of 5X reaction buffer, 2 µl of 10x deoxynucleotide triphosphates (5mM of dCTP, dGTP, dTTP and dATP), 1 µl of 100 Mm DTT, 5 µl of (α-ATP) (3000Ci/mmol, Amersham biosciences) and 2 µl of Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (200 u/ µl,Invitrogen) were added to each template. After incubation at 50°C for 120 minutes, the labelled cDNA probes were purified from unincorporated p32-nucleotides through the nucleospin extraction (BD biosciences, Clontech).

Microarray hybridization and data analysis

The purified cDNA probes were separately prehybridized to identical Atlas Human 1.2 array membranes at 68°C for 30 minutes using ExpressHyp solution (BD Bioscience Clontech). Briefly, equal concentrations of radiolabeled probes (20x10^6 cpm) were heat-denaturated and added to hybridization buffer containing 1mg of sheared salmon tests DNA (Sigma Alderish). The final probe concentration was 2.5x10^6 cpm/ml hybridization buffer. To remove the non-hybridized probes, membranes were washed four times for 30 minutes each in 2x SSC, 1% SDS at 68 °C, once with 0.1x SSC and 0.5 SDS for 30 minutes at 68 C and then briefly rinsed with 2 x SSC at room temperature. The radioactive signals were visualized by multiple autoradiographic exposures of variable lengths on Kodak biomax films with the corresponding BioMax MS intensifying screen at – 80 °C. The

intensity of each signal was quantified using the Atlas Image 1.0 software. The program assigns a background subtracted intensity value to each spot. A gene was defined as expressed if its background subtracted intensity was greater than 2-fold background. Values were also corrected for differences in the hybridization efficiency between the two membranes by dividing the average expression of all genes in the respective array (global normalization). Adjusted values with relative differences in gene expression more than two up or down were selected.

Cardiac myocytes culture

Human adult cardiac myocytes (HCM) from ScienCell research laboratories were grown in cardiac myocyte medium (CMM). Cells were grown in CMM supplemented with 10 % fetal bovine serum and 1% of penicillin/streptomycin in 5% CO_2 at 37 °C. Cells were grown to approximately 60% confluency, and then the growth medium was replaced with unsupplemented CMM for 48 h before treatment. Cells were treated with 100 µg of *S. aureus* RNA for 12 h or left untreated. All pipettes, plates, and other equipment used for preparation, or culturing of cardiac myocytes were endotoxin-free and disposable.

Results and Discussion

Pathogens contain specific PAMPs, which activate PRRs of the innate immune system. Recently, it has been shown that TLR3, 7, 8, and 9 mediate the recognition of ssRNA (Akira and Hemmi, 2003; Bauer *et al.,* 2008; Takeuchi and Akira, 2007). Bacterial RNA can form PAMPs and serving as a danger signal. Although its immunostimulatory potential is well established, only a few proteins and a limited number of cell lines have been investigated in response to ssRNA. Therefore, the exact effect of bacterial RNA on overall gene expression and the ability of the cell to sense and translate collectively this information into an appropriate response are not established. There is a clear evidence of how immune cells sense danger signals of foreign RNA and accordingly respond, but we know less about the initiation and development of such process in cardiac myocytes. To date, most of the studies reported with single-stranded RNA as agonists of TLR7/8 used cationic lipids or polymers for both in vitro and in vivo studies to provide nuclease stability (Lan *et al.,* 2007; Scheel *et al.,* 2004). However, the toxicity of these formulations and non-specific immune activation is still an obstacle to these applications (Karmali and Chaudhuri, 2007).

Already authors reported that the natural bacterial DNA and RNA derived from pathogenic clinical *S. aureus* and *E. coli* isolates induced cardiac depression without lipid carriers (Paladugu *et al.,* 2004). Very recently, spontaneous uptake of exogenous deoxynucleotides into cardiac myocytes has been reported (Knuefermann *et al.,* 2008). Based on these data and considerations, we decided to exogenously stimulate the human cardiac myocytes with bacterial RNA. Cardiac cells were left untreated or incubated in the presence of 100 µg/ml of *S. aureus* RNA. We used the 12 h time point to explore the possibility that long-term changes in gene expression profiles are important in cardiac response to bacterial RNA. The data presented in this work are the average of three individual experiments repeated under the same experimental conditions and total RNAs were harvested from five cardiac samples treated with or without bacterial RNA. To study responses to intermittent exposure to bacterial RNA, we compared the cardiac gene expression levels using cDNA microarrays represents 1176 genes. In response to pathophysiological stresses, cardiac myocytes may undergo hypertrophic growth or apoptosis, responses associated with the development of cardiac pathologies. There has been much effort expended in gaining an understanding of the stimuli which promote these responses, and in identifying the intracellular signaling pathways which are activated and potentially involved. However, previous studied have reported that the variability in cellular response observed at the gene expression level could be caused by kind of stressor, stimulation period, and the cell system.

Table.1 The list of up-regulared cardiac genes in response to bacterial RNA. The radioactive signals were visualized by multiple autoradiographic exposures of variable lengths on Kodak biomax films with the corresponding BioMax MS intensifying screen at – 80°C. The intensity of each signal was quantified using the AtlasImage 1.0 software

Gene code	Intensity 1	Background 1	Adjusted Intensity 1	Intensity 2	Background 2	Adjusted Intensity 2	Gene Expression Ratios	Protein/gene
A09b	5200	0	5200	21244	0	13325	2.5625	c-myc purine-binding transcription factor puf; nucleoside diphosphate kinase B (NDP kinase B; NDK B) + nm23-H2S
A01l	1489	0	1489	3520	0	2979	2.000672	CDC25B; CDC25HU2; M-phase inducer phosphatase 2
A04d	59	0	59	298	0	252	4.271186	jun-D
A11d	202	0	202	836	0	707	3.5	v-erbA related protein (EAR3); COUP transcription factor (COUP-TF)
A14l	1942	0	1942	9581	0	8109	4.175592	RCL growth-related c-myc-responsive gene
A01c	177	0	177	2301	0	2065	11.666667	LUCA2; lysosomal hyaluronidase 2 (HYAL2) ; PH-20 homolog
A10f	223	0	223	2142	0	1881	8.434978	shb proto-oncogene
A01f	474	0	474	3940	0	3637	7.672996	vascular endothelial growth factor receptor 1 (VEGFR1); tyrosine-protein kinase receptor flt + soluble VEGFR; tyrosine-protein kinase receptor SFLT
A04l	705	0	705	14587	0	13468	19.10354	DNA-binding protein inhibitor ID-1; Id-1H
A06l	1001	0	1001	2689	0	2482	2.479521	helix-loop-helix protein HLH 1R21; DNA-binding protein inhibitor Id-3; HEIR-1
A02d	321	0	321	1720	0	1487	4.632399	erythroblastosis virus oncogene homolog 1 (ETS-1); p54
A02f	76	0	76	698	0	603	7.93421	tyrosine-protein kinase receptor tyro3 precursor; tyrosine-protein kinase rse; tyrosine-protein kinase sky; tyrosine-protein kinase dtk
A03b	888	0	888	8910	0	7703	8.674549	EB1 protein
A04e	4	0	4	78	0	67	16.75	A-raf proto-oncogene serine/threonine-protein kinase; PKS2
A09d	12	0	12	198	0	171	14.25	ets-related protein tel; ets translocation variant 6 (ETV6)
A13f	114	0	114	392	0	338	2.964912	CBL-B
A13k	4	0	4	24	0	20	5	sprouty 2 (SPRY2)

B09h	128	0	128	4364	0	4029	31.47656	MAP kinase-activated protein kinase 2 (MAPKAP kinase 2; MAPKAPK-2)
B10j	193	0	193	1867	0	1580	8.186528	serine/threonine-protein kinase PCTAIRE 1 (PCTK1)
B01e	2142	0	2142	7245	0	6503	3.035948	urokinase-type plasminogen activator receptor GPI-anchored form precursor (U-PAR); monocyte activation antigen MO3; CD87 antigen
B10m	71	0	71	445	0	399	5.619718	ras-related protein RAB-7
B12h	1919	0	1919	7727	0	6936	3.614383	dual specificity mitogen-activated protein kinase kinase 3 (MAP kinase kinase 3; MAPKK 3; MKK3); ERK activator kinase 3; MAPK/ERK kinase 3 (MEK3)
B14d	35	0	35	258	0	231	6.6	ephrin type-A receptor 1 precursor; tyrosine-protein kinase receptor eph
B14i	728	0	728	2297	0	2062	2.832417	cAMP-dependent protein kinase alpha-catalytic subunit (PKA C-alpha)
B14f	1006	0	1006	13975	0	12277	12.20377	growth factor receptor-bound protein 2 (GRB2) isoform; GRB3-3; SH2/SH3 adaptor GRB2; ASH protein + epidermal growth factor receptor-bound protein 2 (EGFRBP-GRB2)
B03i	64	0	64	742	0	685	10.70312	protein kinase C alpha polypeptide (PKC-alpha; PKCA)
B05j	2770	0	2770	8691	0	8024	2.896751	c-jun N-terminal kinase 2 (JNK2); JNK55
B13k	13	0	13	39	0	36	2.769231	cAMP-dependent protein kinase type II beta regulatory subunit (PRKAR2B; PKR2)
B02b	19	0	19	1708	0	1476	77.68421	sodium/hydrogen exchanger 1 (Na+/H+ exchanger 1; NHE1); amiloride-sensitive Na+/H+ antiporter
B02j	61	0	61	282	0	243	3.983607	Janus kinase 1 (JAK1)
B04h	19	0	19	306	0	264	13.89473	tyrosine-protein kinase ack
B05i	112	0	112	1640	0	1417	12.65178	protein kinase C delta (NPKC-delta)
B05j	4921	0	4921	12178	0	10528	2.139403	c-jun N-terminal kinase 2 (JNK2); JNK55
B06j	162	0	162	608	0	525	3.240741	C-jun N-terminal kinase 3 alpha2 (JNK3A2); PRKM10 + MAP kinase p493F12
B07i	16	0	16	366	0	316	19.75	protein kinase C eta type (NPKC-eta); PKC-L
B11h	1096	0	1096	17880	0	15458	14.10401	dual specificity mitogen-activated protein kinase kinase 2 (MAP kinase kinase 2; MAPKK 2); ERK activator kinase 2; MAPK/ERK kinase 2

								(MEK2)
B12j	139	0	139	3960	0	3423	24.62589	tyrosine-protein kinase tec
B13h	80	0	80	5714	0	4940	61.75	c-jun N-terminal kinase kinase 1 (JNKK); JNK activating kinase 1 (JNKK1); MAP kinase kinase 4 (MKK4)
C05n	60	0	60	736	0	622	10.366667	6-O-methylguanine-DNA methyltransferase (MGMT); methylated-DNA-protein-cysteine methyltransferase
C08e	22	0	22	168	0	142	6.454545	tuberin; tuberous sclerosis 2 protein (TSC2)
C11k	410	0	410	4660	0	3944	9.619513	ALG-2 calcium-binding protein
C12d	294	0	294	1895	0	1604	5.455782	cortactin; amplaxin; ems-1 oncogene
C08n	124	0	124	917	0	823	6.637097	xeroderma pigmentosum group C repair complementing protein p58/HHR23B
C09j	533	0	533	3727	0	3345	6.275797	IEX-1L anti-death protein; PRG-1; DIF-2
C06g	81	0	81	1164	0	1074	13.25925	tumor necrosis factor receptor 1 (TNFR1); tumor necrosis factor binding protein 1 (TBP1); CD120A antigen
C01g	2	0	2	20986	0	18143	9071.5	retinoic acid receptor beta (RXR-beta; RXRB)
D05m	586	0	586	3291	0	2785	4.75256	nuclear factor NF-kappa-B p100 subunit; nuclear factor NF-kappa-B p52 subunit; H2TF1; oncogene lyt-10
D08i	245	0	245	659	0	557	2.273469	major prion protein precursor (PRP); PRP27-30; PRP33-35C; ASCR
D10j	204	0	204	854	0	722	3.539216	ets domain protein elk-3; NET; SRF accessory protein 2 (SAP2)
D14a	8	0	8	52	0	46	5.75	deoxyribonuclease II (DNase II); acid DNase; lysosomal DNase II
D02m	38	0	38	1884	0	1655	43.55263	E2F dimerization partner 1; DRTF1-polypeptide 1 (DP1)
D03n	357	0	357	7644	0	6715	18.80952	cellular nucleic acid binding protein (CNBP); sterol regulatory element-binding protein
D06k	234	0	234	1904	0	1672	7.145299	transcription repressor protein PRDI-BF1; beta-interferon gene positive regulatory domain I binding factor; BLIMP1
D05j	1082	0	1082	25222	0	23287	21.52218	hepatocyte nuclear factor 4 (HNF4); transcription factor 14
D03m	107	0	107	2222	0	1921	17.95327	interferon regulatory factor 2 (IRF2)
D05k	19	0	19	1054	0	911	47.94736	microphthalmia-associated transcription factor (MITF)

D06l	640	0	640	1766	0	1526	2.384375	transcription factor GATA-4; GATA binding factor-4
D06n	42	0	42	172	0	148	3.523809	transcriptional repressor NF-X1
D07l	196	0	196	630	0	544	2.77551	glucocorticoid receptor repression factor 1
D09l	162	0	162	770	0	665	4.104939	interferon regulatory factor 7 (IRF-7)
E03e	522	0	522	1465	0	1240	2.375479	heat shock factor protein 1 (HSF1); heat shock transcription factor 1 (HSTF1); TCF5
E03k	95	0	95	336	0	284	2.989474	neuromedin B receptor (NMBR); neuromedin-B-preferring bombesin receptor
E06e	2802	0	2802	6662	0	5639	2.012491	DNA-binding protein TAXREB302; albumin D box-binding protein (DBP)
E07d	12	0	12	41	0	34	2.833333	orphan hormone nuclear receptor
E07e	528	0	528	1269	0	1074	2.034091	zinc finger protein 91 (ZNF92); HPF7; HTF10
E10k	138	0	138	2755	0	2331	16.89130	corticotropin releasing factor receptor 1 precursor (CRF-R; CRF1)
E06i	2479	0	2479	5815	0	5220	2.105688	integrin alpha 3 (ITGA3); galactoprotein B3 (GAPB3); VLA3 alpha subunit; CD49C antigen
E07b	272	0	272	654	0	587	2.158088	fli-1 oncogene; ergB transcription factor
E10k	7194	0	7194	19600	0	17595	2.445788	corticotropin releasing factor receptor 1 precursor (CRF-R; CRF1)
E02c	1348	0	1348	5786	0	5083	3.770772	R kappa B DNA-binding protein
E04c	731	0	731	3669	0	3223	4.409029	nuclear factor NF45
E08i	2929	0	2929	16914	0	14859	5.073062	fibronectin receptor alpha subunit (FNRA); integrin alpha 5 (ITGA5); VLA5; CD49E antigen
E01e	124	0	124	871	0	804	6.483871	CCAAT displacement protein; CUTL1; CASP
E02c	883	0	883	9179	0	8475	9.597961	R kappa B DNA-binding protein
E03k	1408	0	1408	21450	0	19805	14.06605	neuromedin B receptor (NMBR); neuromedin-B-preferring bombesin receptor
E07f	5400	0	5400	14081	0	13001	2.407593	RPD3 protein; histone deacetylase 1 (HD1)
E08g	225	0	225	3620	0	3342	14.85333	integrin alpha E precursor (ITGAE); mucosal lymphocyte-1 antigen; hml-1 antigen; CD103 antigen
E07g	12	0	12	178	0	153	12.75	intercellular adhesion molecule 2 precursor (ICAM2); CD102 antigen
E12g	141	0	141	1574	0	1360	9.645391	vitronectin receptor alpha subunit (VNRA); integrin alpha 5 subunit (ITGA5); CD51 antigen

F05n	72	0	72	15928	0	9990	138.75	metalloproteinase inhibitor 1 precursor (TIMP1); erythroid potentiating activity (EPA); fibroblast collagenase inhibitor
F06n	972	0	972	3336	0	2092	2.152263	TIMP-2 (MI)
F10g	4644	0	4644	23628	0	14820	3.191215	macrophage inflammatory protein 2 alpha (MIP2-alpha); growth-regulated protein beta (GRO-beta)
F12g	14416	0	14416	46980	0	29468	2.044118	granulocyte chemotactic protein 2 (GCP 2); neutrophil-activating peptide ENA-78
F14g	2996	0	2996	26720	0	16760	5.594125	interleukin-8 precursor (IL-8); monocyte-derived neutrophil chemotactic factor (MDNCF); T-cell chemotactic factor; neutrophil-activating protein 1 (NAP1); lymphocyte-derived neutrophil-activating factor (LYNAP); protein 3-10C
F02a	3	0	3	235	0	198	66	heat shock cognate 71-kDa protein
F02n	96	0	96	239	0	202	2.104167	protein C inhibitor (PROCI; PCI); plasma serine protease inhibitor precursor; plasminogen activator inhibitor 3 (PLANH3; PAI3)
F03e	112	0	112	391	0	330	2.946429	macrophage-specific colony-stimulating factor (CSF-1; MCSF)
F06d	407	0	407	3989	0	3376	8.29484	insulin-like growth factor II (IGF2); somatomedin A
F07e	671	0	671	3729	0	3156	4.703428	neuroleukin (NLK); glucose-6-phosphate isomerase (GPI); phosphoglucose isomerase (PGI); phosphohexose isomerase (PHI)
F11e	1152	0	1152	3348	0	2833	2.459201	macrophage inflammatory protein 1 alpha precursor (MIP1-alpha); tonsillar lymphocyte LD78 alpha protein; G0S19-1 protein; PAT 464.2; SIS-beta; small inducible cytokine A3 (SCYA3)
F11f	35	0	35	3366	0	2849	81.40000	endothelin 2 (ET2)
F12f	3813	0	3813	28667	0	24265	6.363756	hepatocyte growth factor-like protein; macrophage-stimulating protein (MSP)
F13i	132	0	132	3378	0	2859	21.65909	interleukin-6 precursor (IL-6); B-cell stimulatory factor 2 (BSF-2); interferon beta-2 (IFNB2); hybridoma growth factor
F08a	209	0	209	2146	0	1926	9.215311	thioredoxin peroxidase 2 (TDPX2); thioredoxin-dependent peroxide reductase 2; proliferation-associated gene (PAG); natural killer cell

								enhancing factor A (NKEFA)
F11d	31	0	31	6717	0	6029	19.448	granulocyte colony-stimulating factor precursor (G-CSF); pluripoietin; CSF3
F13b	3750	0	3750	8567	0	7690	2.050667	glutathione S-transferase M1
F13m	793	0	793	8640	0	7756	9.780581	cathepsin D precursor (CTSD)
F14b	1080	0	1080	5419	0	4864	4.503704	liver glutathione S-transferase subunit 1
F14k	257	0	257	4496	0	4036	15.70428	proteasome inhibitor HPI31 subunit
F02b	2240	0	2240	10543	0	9262	4.134821	heat-shock protein 40 (HSP40)
F04j	3417	0	3417	9266	0	8140	2.382207	interleukin-13 precursor (IL-13); NC30
F07b	12243	0	12243	32413	0	28475	2.325819	cytosolic superoxide dismutase 1 (SOD1)
F13b	2817	0	2817	17813	0	15649	5.555201	glutathione S-transferase M1
F13i	1759	0	1759	16215	0	14245	8.098351	interleukin-6 precursor (IL-6); B-cell stimulatory factor 2 (BSF-2); interferon beta-2 (IFNB2); hybridoma growth factor
F08a	2462	0	2462	12734	0	11757	4.775386	thioredoxin peroxidase 2 (TDPX2); thioredoxin-dependent peroxide reductase 2; proliferation-associated gene (PAG); natural killer cell enhancing factor A (NKEFA)
F08g	376	0	376	6013	0	5551	14.76329	leukemia inhibitory factor precursor (LIF); differentiation-stimulating factor (D factor); melanoma-derived LPL inhibitor (MLPLI); HILDA
F10a	2849	0	2849	12747	0	11769	4.130923	dioxin-inducible cytochrome P450 1B1 (CYP1B1)
F12c	608	0	608	3242	0	2993	4.922698	bone morphogenetic protein 1 (BMP1) + procollagen C-proteinase (pCP-2)
F02b	4840	0	4840	21500	0	18587	3.840289	heat-shock protein 40 (HSP40)
F02m	78	0	78	1570	0	1357	17.39743	matrix metalloproteinase 1 (MMP1); interstitial collagenase precursor (CLG); fibroblast collagenase
F06j	1354	0	1354	23688	0	20479	15.124815	interleukin-11 (IL-11); adipogenesis inhibitory factor (AGIF)
F08g	1174	0	1174	9578	0	8280	7.052811	leukemia inhibitory factor precursor (LIF); differentiation-stimulating factor (D factor); melanoma-derived LPL inhibitor (MLPLI); HILDA

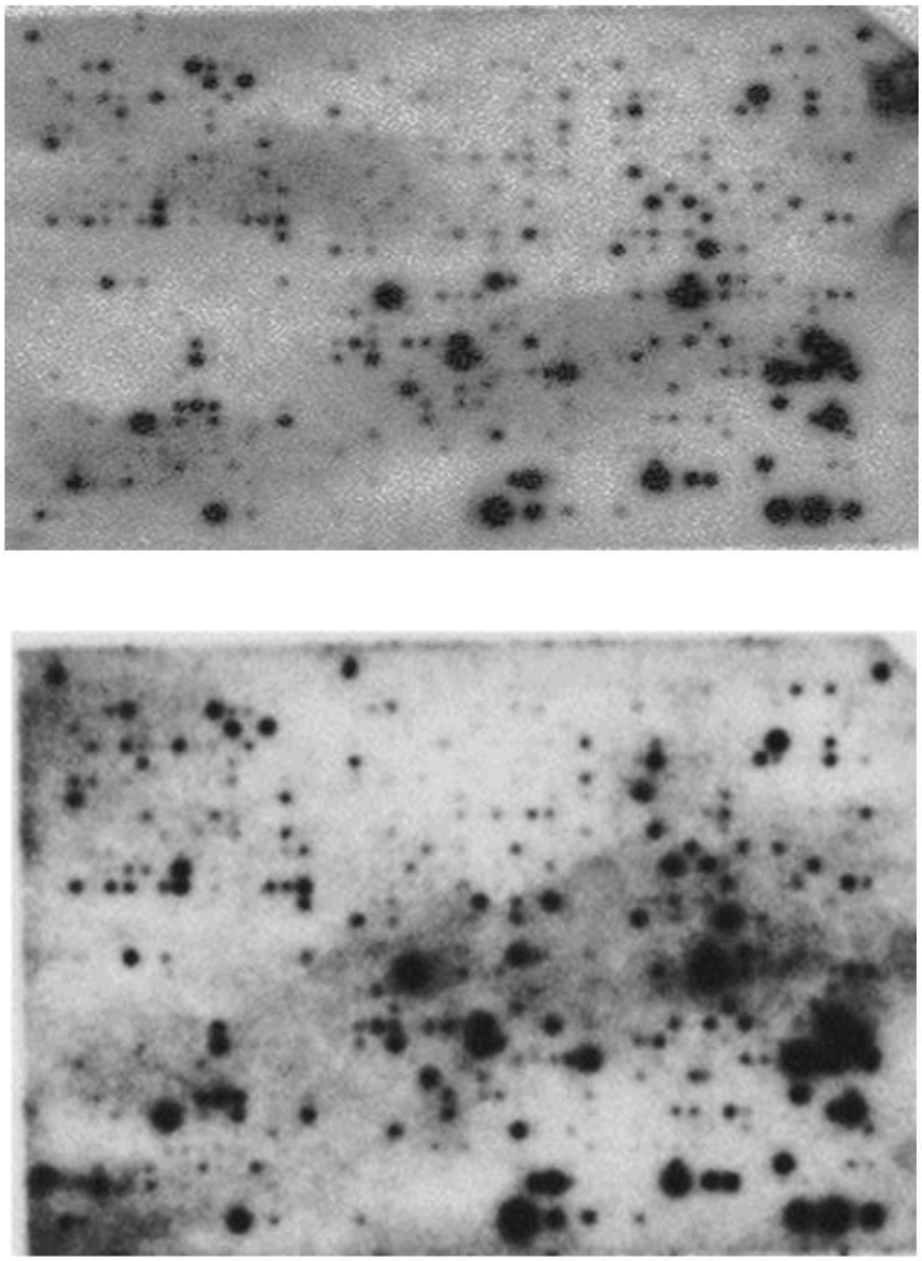
Table.2 The list of down-regulared cardiac genes in response to bacterial RNA. The radioactive signals were visualized by multiple autoradiographic exposures of variable lengths on Kodak biomax films with the corresponding BioMax MS intensifying screen at –80°C. The intensity of each signal was quantified using the AtlasImage 1.0 software

Gene code	Intensity 1	Background 1	Adjusted Intensity 1	Intensity 2	Background 2	Adjusted Intensity 2	Gene Expression Rations	Protein/gene
A07d	3023	0	3023	9987	0	11798	3.902746	fos-related antigen (FRA1)
A13g	11	0	11	105	0	124	11.272727	cyclin K
A03l	853	0	853	7068	0	7873	9.229777	prothymosin alpha (ProT-alpha; PTMA)
A04b	7976	0	7976	16530	0	18413	2.308551	ezrin; cytovillin 2; villin 2 (VIL2)
A10b	2331	0	2331	7472	0	8323	3.57057	nucleoside diphosphate kinase A (NDK A); NDP kinase A; tumor metastatic process-associated protein; metastasis inhibition factor NM23 (NM23-H1)
A12l	1220	0	1220	3116	0	3471	2.845082	transducer of ERBB2 (TOB)
A14j	1041	0	1041	8463	0	9427	9.055716	cyclin-dependent kinase regulatory subunit (CKS2)
A09h	496	0	496	2817	0	3206	6.46371	fte-1; yeast mitochondrial protein import homolog; 40S ribosomal protein S3A (RPS3A)
A13l	12922	0	12922	22995	0	26174	2.025538	p55CDC
A07j	17	0	17	445	0	481	28.29411	p35 cyclin-like CAK1-associated protein
A07e	26	0	26	50	0	57	2.192308	C-kit proto-oncogene; mast/stem cell growth factor receptor precursor (SCFR); CD117 antigen
B01n	373	0	373	3311	0	3911	10.48525	ADP-ribosylation factor 1
B04k	119	0	119	1820	0	2027	17.03361	protein kinase MLK-3; sprk
B10n	1196	0	1196	2463	0	2743	2.293478	guanine nucleotide-binding protein G-i/G-s/G-t beta subunit 2; transducin beta 2 subunit 2
B01k	400	0	400	7218	0	8348	20.87000	lipid-activated protein kinase PRK1;PKN cell morphology-related protein kinase

B02k	18	0	18	338	0	390	21.66666	serum- & glucocorticoid-regulated serine/threonine protein kinase (SGK)
B02l	12	0	12	105	0	121	10.08333	myotonic dystrophy protein kinase-like protein
B03l	92	0	92	1390	0	1607	17.46739	ribosomal protein S6 kinase II alpha 1 (S6KII-alpha 1); ribosomal S6 kinase 1 (RSK1)
B04l	436	0	436	1696	0	1961	4.497706	ribosomal protein S6 kinase II alpha 2 (S6KII-alpha 2); ribosomal S6 kinase 3 (RSK3)
B05l	282	0	282	972	0	1124	3.985816	ribosomal protein S6 kinase II alpha 3 (S6KII-alpha 3); ribosomal S6 kinase 2 (RSK2); insulin-stimulated protein kinase 1 (ISPK1)
C03m	638	0	638	2372	0	2802	4.39185	MCM5 DNA replication licensing factor; CDC46 homolog
C09d	25017	0	25017	45055	0	53227	2.127633	rho GDP dissociation inihibitor 1 (RHO-GDI 1); RHO-GDI alpha (GDIA1); ARHGDIA
C10a	775	0	775	2377	0	2647	3.415484	serine/threonine protein phosphatase 2B catalytic subunit alpha isoform; calmodulin-dependent calcineurin A subunit alpha isoform; CAM-PRP catalytic subunit
C08K	30	0	30	88	0	101	3.366667	ionizing radiation resistance-conferring protein + death-associated protein 3 (DAP3)
C12e	13536	0	13536	33179	0	38376	2.835106	zyxin + zyxin-2
C14l	346	0	346	1110	0	1283	3.708092	activator 1 37-kDa subunit; replication factor C 37-kDa subunit (RFC37); RFC4
D02m	172	0	172	980	0	1091	6.343023	E2F dimerization partner 1; DRTF1-polypeptide 1 (DP1)
D08m	335	0	335	2754	0	3067	9.155224	endothelial transcription factor GATA2
D03n	2976	0	2976	10120	0	10960	3.682796	cellular nucleic acid binding protein (CNBP); sterol regulatory element-binding

								protein
D04m	68	0	68	160	0	185	2.720588	hepatocyte nuclear factor 4 (HNF4); transcription factor 14
E10a	4516	0	4516	19869	0	23473	5.197742	early growth response protein 1 (hEGR1); transcription factor ETR103; KROX24; zinc finger protein 225; AT225
E11a	3415	0	3415	16532	0	18415	5.392386	transcription factor ETR101
E05e	2458	0	2458	6576	0	7485	3.045159	putative transcription activator DB1
E08h	731	0	731	8511	0	9217	12.60875	CD44 antigen hematopoietic form precursor (CD44H); phagocytic glycoprotein I (PGP-1); HUTCH-I; extracellular matrix receptor-III (ECMR-III); GP90 lymphocyte homing/adhesion receptor (LHR); hermes antigen
E07e	1624	0	1624	5246	0	6067	3.735837	zinc finger protein 91 (ZNF92); HPF7; HTF10
F09g	172	0	172	297	0	350	2.034884	acidic fibroblast growth factor (AFGF) + heparin-binding growth factor 1 precursor (HBGF-1) + beta-endothelial cell growth factor (ECGF-beta)
F07g	132	0	132	6336	0	5916	4.81818	platelet-derived growth factor A subunit precursor (PDGFA; PDGF-1)
F02n	38	0	38	1243	0	1384	36.42105	protein C inhibitor (PROCI; PCI); plasma serine protease inhibitor precursor; plasminogen activator inhibitor 3 (PLANH3; PAI3)
F05e	3666	0	3666	8433	0	9393	2.562193	hepatoma-derived growth factor (HDGF)
F14j	191	0	191	2592	0	11798	14.69633	Wnt-13

Fig.1 Bacterial RNA differentially Modulates Cardiac Gene Expression. The Human adult cardiac myocytes were treated with 100 µg/ml intact *S. aureus* RNA (bottom figure) for 24 hrs or left untreated (top figure). The purified cDNA probes extracted from both experiments were separately prehybridized to identical Atlas Human 1.2 Array Membranes. The figure represents three experiments repeated separately.



Therefore, for some genes we are unable to interpret the observation in a manner related to the function of this change in cardiac expression. Our analysis has revealed changes in the cardiac expression of about 140 genes in response to bacterial RNA. According to Gene Ontology annotation, a large proportion of upregulated genes (about 100 gene) encode proteins involved in regulating the adaptive immune responses including, proinflammatory cytokines and chemokines (Table 1). It should be

noted that a significant number of genes involved in stress signalling and homeostasis has been also upregulated. Among upregulated cardiac, we have identified genes encoding proteins involved in cell survival and homeostasis. We also determined the induction of many inflammation genes that represent the convergence point of numerous signalling pathways. Inflammation is an important component of cardiac pathology associated with a number of heart diseases including sepsis, myocarditis, cardiomyopathy, and myocardial infraction. The immunological mediators have been increasingly though to influence not only the inflammatory molecules but also cardiac function and remodelling (Mitchell *et al.*, 2007).

We also have identified the gene suppression of about 42 cardiac genes. Interestingly, genes implicated in regulation of cardiac cell cycle, cell adhesion and cytoskeleton remodelling, DNA repair, and transcriptional regulation are among these repressed genes. In mammals, cardiac myocytes rapidly proliferate during fetal life but exit the cell cycle soon after birth (Engel, 2005). Although the extent to which adult cardiac myocytes are capable of cell cycle reentry is controversial and species-specific differences may exist, it appears that for the vast majority of adult cardiac myocytes the predominant form of growth postnatally is an increase in cell size (hypertrophy) not number. The loss of cardiomyocytes triggers pathological remodeling, a process resulting in structural changes within the healthy myocardium adjacent to as well as remote from the infarcted area. Myocardial remodeling is characterized by progressive changes in ventricular size, shape, and function which leads to further loss of cardiomyocytes (necrosis and apoptosis) and increase in interstitial fibrosis. One major limitation in the field has been the lack of adequate models or techniques in mammalian systems to truly mechanistically analyze cardiac myocyte proliferation and differentiate cell cycle reentry from its downstream consequences including increased ploidy, nuclear mitosis, cytokinesis, and apoptosis (Engel, 2005; Rubart and Field, 2006; Ahuja *et al.*, 2007).

Our results established other definitive properties of human cardiac myocytes such as sensing and responding to danger signals with a complex molecular response, thus analogous in some respect to immune cells.

Acknowledgement

All authors are equally contributed in performing this work and preparing the manuscript. The financial support and funding of the Laurentian University / Biomolecular Sciences Programme is also gratefully acknowledged.

References

Ahuja, P., Sdek, P. and Maclellan, W. R. (2007). Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev*. 87:521-544.

Akira, S. and Hemmi, H. (2003). Recognition of pathogen-associated molecular patterns by TLR family. *Immunol Lett*. 85:85-95.

Alexopoulou, L. and Kontoyiannis, D. (2005). Contribution of microbial-associated molecules in innate mucosal responses. *Cell Mol Life Sci*. 62:1349-1358.

Bauer, S., Pigisch, S., Hangel, D., Kaufmann, A. and Hamm, S. (2008). Recognition of nucleic acid and nucleic acid analogs by Toll-like receptors 7, 8 and 9. *Immunobiology*. 213:315-328.

Boyd, J. H., Mathur, S., Wang, Y., Bateman, R. M. and Walley, K. R. (2006). Toll-like receptor stimulation in cardiomyoctes decreases contractility and initiates an NF-kappaB dependent inflammatory response. *Cardiovasc Res*. 72:384-393.

Cartwright, N., Mcmaster, S. K., Sorrentino, R., Paul-Clark, M., Sriskandan, S., Ryffel, B., Quesniaux, V. F., Evans, T. W. and Mitchell, J. A. (2007). Elucidation of toll-like receptor and adapter protein signaling in vascular dysfunction induced by gram-positive *Staphylococcus aureus* or gram-negative *Escherichia coli*. *Shock*. 27:40-47.

Deane, J. A. and Bolland, S. (2006). Nucleic acid-sensing TLRs as modifiers of autoimmunity. *J Immunol*. 177:6573-6578.

Diebold, S. S., Massacrier, C., Akira, S., Paturel, C., Morel, Y. and Reis E Sousa, C. (2006). Nucleic

acid agonists for Toll-like receptor 7 are defined by the presence of uridine ribonucleotides. *Eur J Immunol*. 36:3256-3267.

Engel, F. B. (2005). Cardiomyocyte proliferation: a platform for mammalian cardiac repair. *Cell Cycle*. 4:1360-1363.

Erridge, C. (2008). The roles of pathogen-associated molecular patterns in atherosclerosis. *Trends Cardiovasc Med*. 18:52-56.

Frantz, S., Ertl, G. and Bauersachs, J. (2007). Mechanisms of disease: Toll-like receptors in cardiovascular disease. *Nat Clin Pract Cardiovasc Med*. 4:444-454.

Frantz, S., Kobzik, L., Kim, Y. D., Fukazawa, R., Medzhitov, R., Lee, R. T. and Kelly, R. A. (1999). Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. *J Clin Invest*. 104:271-280.

Hamm, S., Heit, A., Koffler, M., Huster, K. M., Akira, S., Busch, D. H., Wagner, H. and Bauer, S. (2007). Immunostimulatory RNA is a potent inducer of antigen-specific cytotoxic and humoral immune response in vivo. *Int Immunol*. 19:297-304.

Karmali, P. P. and Chaudhuri, A. (2007). Cationic liposomes as non-viral carriers of gene medicines: resolved issues, open questions, and future promises. *Med Res Rev*. 27:696-722.

Kawai, T. and Akira, S. (2007). TLR signaling. *Semin Immunol*. 19:24-32.

Knuefermann, P., Schwederski, M., Velten, M., Krings, P., Ehrentraut, H., Rudiger, M., Boehm, O., Fink, K., Dreiner, U., Grohe, C., Hoeft, A., Baumgarten, G., Koch, A., Zacharowski, K. and Meyer, R. (2008). Bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility: role of Toll-like receptor 9. *Cardiovasc Res*. 78:26-35.

Koski, G. K., Kariko, K., Xu, S., Weissman, D., Cohen, P. A. and Czerniecki, B. J. (2004). Cutting edge: innate immune system discriminates between RNA containing bacterial versus eukaryotic structural features that prime for high-level IL-12 secretion by dendritic cells. *J Immunol*. 172:3989-3993.

Lan, T., Kandimalla, E. R., Yu, D., Bhagat, L., Li, Y., Wang, D., Zhu, F., Tang, J. X., Putta, M. R., Cong, Y., Trombino, A. F., Sullivan, T. and Agrawal, S. (2007). Stabilized immune modulatory RNA compounds as agonists of Toll-like receptors 7 and 8. *Proc Natl Acad Sci U S A*. 104:13750-13755.

Mitchell, J. A., Ryffel, B., Quesniaux, V. F., Cartwright, N. and Paul-Clark, M. (2007). Role of pattern-recognition receptors in cardiovascular health and disease. *Biochem Soc Trans*. 35:1449-1452.

Paladugu, B., Kumar, A., Parrillo, J. E., Der, S., Osman, J., Mensing, J., Falvo, L., Xu, X. and Kumar, A. (2004). Bacterial DNA and RNA induce rat cardiac myocyte contraction depression in vitro. *Shock*. 21:364-369.

Rubart, M. and Field, L. J. (2006). Cardiac regeneration: repopulating the heart. *Annu Rev Physiol*. 68:29-49.

Scheel, B., Braedel, S., Probst, J., Carralot, J. P., Wagner, H., Schild, H., Jung, G., Rammensee, H. G. and Pascolo, S. (2004). Immunostimulating capacities of stabilized RNA molecules. *Eur J Immunol*. 34:537-547.

Sioud, M. (2006). Innate sensing of self and non-self RNAs by Toll-like receptors. *Trends Mol Med*. 12:167-176.

Sugiyama, T., Gursel, M., Takeshita, F., Coban, C., Conover, J., Kaisho, T., Akira, S., Klinman, D. M. and Ishii, K. J. (2005). CpG RNA: identification of novel single-stranded RNA that stimulates human CD14+CD11c+ monocytes. *J Immunol*. 174:2273-2279.

Takeuchi, O. and Akira, S. (2007). Signaling pathways activated by microorganisms. *Curr Opin Cell Biol*. 19:185-191.

Takeuchi, O. and Akira, S. (2008). MDA5/RIG-I and virus recognition. *Curr Opin Immunol*. 20:17-22.

How to cite this article:

Farag A. Bleiblo, Souad A. M. Moftah, Omar S. Alqabbasi, Siham R. Agouri, Asma A. Elamari, Samira F. M. Bo Hagar and Aseem Kumar. 2022. Microaaray Analysis of Differential Gene Expression Profiles of Human Adult Cardiac Myocytes Challenged with Non-Self RNA. *Int.J.Curr.Microbiol.App.Sci.* 11(10): 17-32. doi: https://doi.org/10.20546/ijcmas.2022.1110.003