HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

J Immunol. 2016 January 1; 196(1): 182–195. doi:10.4049/jimmunol.1501527.

Alcohol consumption modulates host defense in rhesus macaques by altering gene expression in circulating leukocytes

Tasha Barr[*], **Thomas Girke**[†], **Suhas Sureshchandra**[*], **Christina Nguyen**[*], **Kathleen Grant**[‡], and **Ilhem Messaoudi**[*,§]

[*]Division of Biomedical Sciences, School of Medicine, University of California-Riverside, Riverside, CA 92521, USA

[†]Institute of Integrative Genome Biology, University of California-Riverside, Riverside, CA 92521, USA

[‡]Division of Neurosciences, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR 97006, USA

Abstract

Several lines of evidence indicate that chronic alcohol use disorder leads to increased susceptibility to several viral and bacterial infections whereas moderate alcohol consumption decreases incidence of colds and improves immune responses to some pathogens. In line with these observations, we recently showed that heavy ethanol intake (average blood ethanol concentrations (BECs) >80 mg/dl) suppressed, whereas moderate alcohol consumption (BEC <50 mg/dl) enhanced T and B-cell responses to Modified Vaccinia Ankara (MVA) vaccination in a nonhuman primate model of voluntary ethanol consumption. To uncover the molecular basis for impaired immunity with heavy alcohol consumption and enhanced immune response with moderate alcohol consumption, we performed a transcriptome analysis using PBMCs isolated on day 7 post-MVA vaccination, the earliest time point at which we detected differences in T-cell and antibody responses. Overall, chronic heavy alcohol consumption reduced expression of immune genes involved in response to infection and wound healing, and increased expression of genes associated with the development of lung inflammatory disease and cancer. In contrast, chronic moderate alcohol consumption upregulated expression of genes involved in immune response and reduced expression of genes involved in cancer. In order to uncover mechanisms underlying the alterations in PBMC transcriptomes, we profiled the expression of microRNAs within the same samples. Chronic heavy ethanol consumption altered the levels of several microRNAs involved in cancer and immunity and known to regulate expression of mRNAs differentially expressed in our dataset.

INTRODUCTION

Alcohol use disorder (AUD) results in a significant increase in both incidence and severity of infections such as bacterial pneumonia, tuberculosis, hepatitis C virus, and HIV (1–3).

[§]To whom correspondence should be addressed: Ilhem Messaoudi, PhD, University of California Riverside, 900 University Avenue, Riverside, CA 92521, Tel.: 951-827-7774, Fax: 951-579-4118, ilhem.messaoudi@ucr.edu.

Similarly, chronic ethanol consumption in rodents results in increased pathogen burden and impaired ability to clear *Listeria monocytogenes* (4), *Mycobacterium tuberculosis* (5), and influenza virus (6). Likewise, rhesus macaques given ethanol via intragastric cannula show increased simian immunodeficiency virus replication compared to controls (7). Increased vulnerability to infection in individuals with AUD is due to changes in barrier function as well as innate and adaptive immunity (8). Dysregulation of tight junction proteins in the lungs and gut increases permeability, leading to bacterial translocation into the alveolar space and circulation, respectively (9, 10). In addition, AUD results in the inhibition of phagocytic functions, reduction of chemotaxis and aberrant cytokine production, and diminished lymphocyte numbers and antigen-specific responses (11).

In contrast, data from several studies support a beneficial role for moderate alcohol consumption on immunity. Moderate alcohol consumption is associated with decreased incidence of the common cold in humans (12–14) as well as improved bacterial clearance and increased delayed cutaneous hypersensitivity response following infection with *Mycobacteria bovis* in rats (15). Recently, we showed using a macaque model of ethanol self-administration (16) that moderate consumption resulted in a more robust T-cell and antibody vaccine response to Modified Vaccinia Ankara (MVA), while heavy drinkers generated blunted T-cell and antibody response compared to controls (17). Moreover, we showed that the dose-dependent effects of ethanol on the immune response to the MVA vaccine were independent of changes in frequency of major immune cell subsets. Specifically, numbers of circulating lymphocyte, monocyte, and neutrophil as well as the frequency of CD4 T cell, CD8 T cell, and CD20 B cells (and their naïve and memory subsets) did not differ between control and ethanol consuming animals (17). Instead, we detected changes in the expression of several microRNAs (miRNAs) associated with development and function of the immune system, suggesting that ethanol dose-dependent modulation of immunity is mediated by changes in gene expression. Therefore, in this study, we compared the transcriptomes of PBMCs isolated from controls, moderate, and heavy drinkers on day 7 post-MVA vaccination.

Our results revealed that chronic heavy ethanol consumption was associated with significant downregulation of genes involved in immune response to infection and wound healing as well as upregulation of genes associated with development of obstructive lung disease and cancer. In contrast, chronic moderate alcohol consumption was associated with reduced expression of genes involved in neoplasia and the upregulation of genes involved in host defense. In order to uncover mechanisms underlying the alterations in PBMC transcriptomes, we also examined changes in miRNA expression. Our analysis showed that chronic heavy ethanol consumption altered the expression of several miRNAs whose targets were differentially expressed in our data set and are involved in cancer progression and immune function. Overall, data presented in this manuscript provide novel insight into the mechanisms by which excessive alcohol consumption interferes with immune responses, and exacerbates co-morbidities such as poor wound healing, lung disease, and cancer, while moderate consumption improves immunity.

MATERIALS AND METHODS

Ethics statement

This study was performed in strict accordance with the recommendations detailed in the Guide for the Care and Use of Laboratory Animals of the National Institute of Health, the Office of Animal Welfare and the United States Department of Agricultures. All animal work was approved by the ONPRC Institutional Animal Care and Use Committee (IACUC).

Animal studies and sample description

The animal model and vaccination strategy were previously described (17). Briefly, we used schedule-induced polydipsia to establish reliable self-administration of 4% (w/v) ethanol in 8 male rhesus macaques (16). Four animals served as controls for a total of 12 animals. Following a 4-month induction period, animals were allowed a choice of 4% ethanol or water for 22hr/day every day for 12 months. In this nonhuman primate model of voluntary self-administration, animals segregate naturally into heavy and moderate drinkers within 2–3 months, and these patterns remain stable for at least 12 months (18). In this specific cohort, ethanol-drinking animals segregated into two cohorts, n=4 each based on average blood ethanol (BEC) values: moderate drinkers with average BEC of 22.3–48.8 mg/dl and heavy drinkers with average BEC of 90–126 mg/dl (17). All 12 animals were vaccinated with MVA prior to induction of ethanol and again after 7 months of open access to ethanol. We used PBMCs isolated 7 days after booster vaccination for RNA and microRNA expression analysis. Only 3 animals from each group had sufficient numbers of PBMCs for RNA sequencing.

RNA isolation and mRNA library preparation

Total RNA was isolated from PBMCs using the miRNeasy kit (Qiagen, Valencia, CA). One microgram of RNA was used to generate libraries using the NEBNext Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA). Poly(A)-enriched mRNA was fragmented followed by cDNA synthesis with random hexamers. This product underwent end-repair, adapter ligation, and size selection using AMPure XP beads (Beckman Coulter Inc., Brea, CA) to isolate cDNA templates of 320 nucleotides that were amplified by PCR. Each library was prepared with unique index primers for multiplexing and subjected to single-end 100 bp sequencing on the HiSeq2500 platform (Illumina, San Diego, CA).

Small RNA library preparation

One microgram of total RNA extracted as described above underwent adapter ligation and primer hybridization prior to cDNA synthesis and PCR amplification using the NEBNext Small RNA Library Prep Set for Illumina Kit (New England Biolabs, Ipswich, MA). Size selection was performed with AMPure XP beads (Beckman Coulter Inc., Brea, CA) to isolate cDNA templates of 140 nucleotides. Each library was prepared with unique index primers for multiplexing and subjected to single-end 50 bp sequencing on the HiSeq2500 platform (Illumina, San Diego, CA). We were unsuccessful in generating one library from one of the heavy drinkers.

RNA-Seq analysis

Data analysis was performed with the RNA-Seq workflow module of the *systemPiperR* package available on Bioconductor (19, 20). Quality reports were generated with the *seeFastq* function. RNA-Seq reads were mapped with the splice junction aware short read alignment suite Bowtie2/Tophat2 (21, 22) against the *Macaca mulatta* genome sequence from Ensembl (23). For the alignments, we used default parameters of Tophat2 optimized for mammalian genomes. Raw expression values in form of gene-level read counts were generated with the *summarizeOverlaps* function (24). Here, we counted only reads overlapping exonic regions of genes, discarding reads mapping to ambiguous regions of exons from overlapping genes. Given the non-stranded nature of RNA-Seq libraries, the read counting was performed in a non-strand-specific manner. The RNA sequencing data have been deposited in NCBI's Sequence Read Archive (SRA) under the accession number SRP064253 (http://www.ncbi.nlm.nih.gov/sra). Analysis of differentially expressed genes (DEGs) was performed with the GLM method from the *edgeR* package (25, 26). DEGs were defined as those with a fold change of ≥2 and a false discovery rate (FDR) of ≤0.05. Enrichment analysis of functional annotations was performed to identify significant biological pathways including gene ontology (GO) terms and disease biomarkers using MetaCore™ software (GeneGo, Philadelphia, PA).

Small RNA-Seq analysis

Adaptor contaminations were removed (trimmed) from the reads using the *preprocessReads* function from the *systemPipeR* package. The preprocessed reads were aligned with Bowtie2 (21, 22) against the *Macaca mulatta* genome sequence with settings optimized for miRNA alignments, including tolerance of multiple mappings. Reads overlapping with miRNA gene ranges were counted with the *summarizeOverlaps* function as described above, but in a strand-specific manner. The miRNA gene coordinates, required for this step, were downloaded from miRBase (Release version 19). The small RNA sequencing data have been deposited in NCBI's SRA under the accession number SRP064540 (http://www.ncbi.nlm.nih.gov/sra). Differentially expressed miRNA genes were identified with *edgeR* as described above. TargetScan was used to predict genes for each differentially expressed microRNA with a high context ratio of 0.95. These targets were then compared with our list of differentially expressed genes among the three groups of rhesus macaques. These combinations of differentially expressed mRNA and miRNA were then segregated based on the directions of fold changes.

Gene validation via qRT-PCR

cDNA was synthesized from RNA isolated as above, using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). mRNA expression was determined by quantitative reverse transcription PCR using Taqman primer and probe kits specific for *Macaca mulatta* cDNA sequences and a StepOnePlus instrument (Life Technologies, Grand Island, NY). mRNA expression levels of LYZ (Rh02902590), PTGS2 (Rh02787804), THBS1 (Rh00962902), KLF4 (Rh02847953), TLR4 (Rh01060206), CD14 (Rh03648680), CD163 (Hs00174705), and FN1 (Rh02621780) for each sample were calculated relative to control RPL32 mRNA expression using ΔCt calculations.

RESULTS

Heavy alcohol consumption leads to significant changes in gene expression

There were 514 DEGs between controls and heavy drinkers (C7-H7), 479 of which were annotated with 356 downregulated and 123 upregulated genes with heavy drinking (Supplementary Table I). We identified 368 DEGs between moderate and heavy drinkers (M7-H7), 347 of which were annotated with 290 downregulated and 57 upregulated genes with heavy drinking (Supplementary Table II). Finally, of the 60 DEGs between controls and moderate drinkers (C7-M7), 47 were annotated with 29 downregulated and 18 upregulated with moderate ethanol consumption (Supplementary Table III, Fig. 1A).

Heavy ethanol consumption was associated with the largest changes in gene expression compared to both controls and moderate consumption (Fig. 1A, 1B), with 171 downregulated (Fig. 1C), and 26 upregulated DEGs compared to controls and moderate drinkers (Fig. 1D). The overwhelming majority of the downregulated (Fig. 1E) and upregulated (Fig. 1F) DEGs showed a 2–4 fold-change in expression. To confirm the RNA-Seq results, 8 genes differentially expressed with chronic heavy ethanol consumption (LYZ, PTGS2, THBS1, KLF4, TLR4, CD14, CD163, and FN1) were selected for confirmation using qRT-PCR. Changes in expression level of all 8 DEGs were confirmed (Supplementary Fig. 1). In order to better understand the biological relevance of these gene expression changes, we conducted functional enrichment analysis using the MetaCore™ pathway-mapping tool.

Heavy alcohol consumption downregulates genes that promote host defense compared to both moderate drinkers and controls

Of the 171 genes repressed in H7 compared to C7 and M7, 114 mapped to the following GO terms: response to stress, response to wounding, inflammatory response, response to lipid, and positive regulation of response to external stimulus (Fig. 2A). Several DEGs mapping to “response to stress” encode microbial sensors, notably formyl peptide receptor 2 (FPR2, FC=128.2), TLR4 (FC=5.6), CD14 (FC=3.8), pyrin domain-containing-3 (NLRP3, FC=3.3), TLR8 (FC=2.9), and nucleotide-binding oligomerization domain-containing-2 (NOD2, FC=2.4). Other DEGs encode immune receptors such as cadherin EGF LAG seven-pass G-type receptor-1 (CELSR1, FC=30.6), syndecan-2 (SDC2, FC=7.8), plasminogen activator receptor (PLAUR, FC=6.5), macrophage scavenger receptor-1 (MSR1, FC=5.7), IL-1 receptor type-1 (IL1R1, FC=5.7), IL-13 receptor alpha-1 (IL13RA1, FC=4.8), CCR1 (FC=4.5), and neuropilin-1 (NRP1, FC=3.4). Additional DEGs encode chemokines, cytokines, growth factors, and antimicrobial peptides, including matrix metalloproteinase-1 (MMP1, FC=8.6), CXCL8 (FC=7.5), oncostatin-M (OSM, FC=4.4), IL-1β (FC=3.7), vascular endothelial growth factor-A (VEGFA, FC=5.2), heparin-binding EGF-like growth factor (HBEGF, FC=3.4), and S100 calcium binding protein-8/9 (S100A8/9, FC =3.6/2.9).

Several genes listed above also mapped to “response to wounding” and “inflammatory process” and play a role in wound healing (Fig. 2B). For instance, CELSR1, SDC2, VEGFA, HBEGF, and NRP1 promote wound closure (27, 28), while NOD2, NLRP3, CD14, coagulation factor plasminogen activator inhibitor-2 (SERPINB2, FC=17.6), complement

component 5a receptor-1 (C5AR1, FC=4.0) and 3a receptor-1 (C3AR1, FC=2.9), and IL-1β (which activates CXCL8 FC=7.5) promote chemotaxis and leukocyte extravasation into injury sites (29–32).

Additional analysis showed that 89 genes map to these disease categories: obstructive lung diseases, pathologic processes, hypersensitivity, bacterial infection and mycoses, and inflammation (Fig. 2C). Of the 52 genes mapping to “obstructive lung diseases” (Fig. 2D), 24 interact with each other (Fig. 2E), and are important for lung homeostasis, notably MMP1 (FC=8.6, involved in lung alveolar epithelial cell migration (33)), VEGFA (important for alveolar structure (34)), cathelicidin antimicrobial peptide (CAMP/LL37, FC=2.8), and aryl hydrocarbon receptor (AHR, FC=2.7, regulates apoptosis of lung epithelial cells (35)). DEGs mapping to “pathological processes” include Annexin-2 receptor (ANXA2R, FC=17.1), cysteine-rich secretory protein LCCL domain-containing-2 (CRISPLD2, FC=9.7), epiregulin (EREG, FC=9.2), triggering receptor expressed on myeloid cells-1 (TREM1, FC=4.7), and HBEGF, which are involved in protection against cancer, sepsis, endotoxin shock, and necrotizing enterocolitis (36–40).

Heavy drinking downregulates genes that promote wound healing and protect against chronic disease compared only to controls

Of the 185 DEGs repressed in H7 compared to C7, 128 mapped to these GO terms: response to wounding, regulation of response to stimulus, positive regulation of response to stimulus, response to stimulus, and system development (Fig. 3A). The 37 genes mapping to “response to wounding” play an important role in wound healing (Fig. 3B) including diacylglycerol kinase (DGKH, FC=5.8, regulates fibroblast migration (41)), catenin alpha-1 (CTNNA1, FC=3.4, promotes wound repair in bronchial epithelial cells (42)), metallopeptidase inhibitor-2 (TIMP2, FC=3.4, involved in wound closure (43)), solute carrier family-11 (SLC11A1, FC=2.2, regulates macrophage activation in cutaneous wounds (44)), CSF1 (FC=2.1, involved in neoangiogenesis (45)), and hepatocyte growth factor (HGF, FC=2.0, accelerates wound re-epithelialization (28)). One highly downregulated gene mapping to “response to stimulus” is eosinophil peroxidase (EPX, FC=22.6), a potent toxin for bacteria and parasites (46).

Further analysis showed 106 genes mapped to these disease categories: obstructive lung diseases, pathological processes, immune system diseases, bronchial diseases, and immediate hypersensitivity (Fig. 3C). Genes in “obstructive lung diseases” play an important role in lung function (Fig. 3D). For instance, IL-1 receptor-like-1 (IL1RL1, FC=9.2), TLR5 (FC=6.0), arachidonate 15-lipoxygenase (ALOX15, FC=5.5), TREM2 (FC=2.6), and TNF-receptor superfamily-1A (TNFRSF1A, FC=2.0) promote host defense against bacterial infection in the lungs and regulate lung inflammation, whereas HGF (FC=2.0) promotes lung regeneration after injury (28). Polymorphisms in myeloperoxidase (MPO, FC=7.4) and A Disintegrin and Metalloproteinase domain-12 (ADAM12, FC=4.5) modulate the development of lung cancer (47, 48).

Several DEGs that mapped to “immune system diseases” also mapped to “obstructive lung diseases” and “pathological processes” including C-type lectin domain family-10A (CLEC10A, FC=4.8) and ATP-binding cassette subfamily-C-2/3 (ABCC2/3, FC=2.9/3.2),

which play a role in antigen recognition and presentation (49, 50); as well as EGF-like module receptor-1 (EMRI, FC=3.7) and interleukin-12 receptor-beta-2 (IL12Rβ2, FC=2.8), which are critical for host defense (51). Genes unique to this category (Fig. 3E) regulate inflammation such as leucine-rich repeat-containing-18 (LRRC18, FC=104.5) and hemoglobin subunit gamma-2 (HBG2, FC=8.9 (52, 53)); as well as lymphocyte proliferation and differentiation including SH2B adaptor-3 (SH2B3, FC=2.0 (54)) and killer cell lectin-like receptor subfamily-G1 (KLRG1, FC=2.4 (55)).

Heavy alcohol consumption reduces expression of genes that regulate the immune system compared to moderate consumption only

Of the 119 genes downregulated in H7 compared to M7, 66 mapped to the following GO terms: immune system processes, response to stress, immune response, defense response, and regulation of immune system processes (Fig. 4A). In total, 47 mapped to immune system-related GO terms, 20 of which interact with each other (Fig. 4B). DEGs mapping to these GO terms are involved in: 1) lymphocyte activation and recruitment (CXCL10, FC=17.3; SLC16A1, FC=3.2 (56); sterile alpha-motif-domain Src homology-domain nuclear localization signals-1, SAMSN1, FC=3.7 (57); ICAM1, FC=5.5; CD83, FC=3.0 (58)); 2) antimicrobial response (TNFα, FC=7.9; ficolin-2, FCN2, FC=6.5 (59); MD2, FC=2.8); and 3) regulation of gene expression (v-ets avian erythroblastosis virus oncogene homolog-2, ETS2, FC=2.5; B-cell lymphoma 2-related protein-A1, BCL2A1, FC=5.4 (60); B-cell CLL/lymphoma-3/6, BCL3/6, FC=2.5/2.0 (61, 62); Kruppel-like factor-10, KLF10, FC=3.0 (63); nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha, NFKBIA, FC=2.9 (64)).

Several genes mapping to “immune system process” (Fig. 4C) also map to “response to stress”, notably hypoxia-inducible factor-1-alpha (HIF1A, FC=3.0) that regulates expression of genes that counter oxidative stress and ETS2, which is induced by shear stress to preserve integrity of microvascular walls (65). Additional notable DEGs that only mapped to “response to stress” included Gadd45-gamma (GADD45G, FC=8.4, important in anti-tumor immune responses (66)) and BMX non-receptor tyrosine kinase (BMX, FC=11.5, promotes tight junction formation in epithelial cells during chronic hypoxia (67)).

Heavy drinking increases expression of genes associated with impaired wound healing, cardiovascular disease and cancer

Within the 26 genes upregulated in H7 compared to both C7 and M7 (Fig. 5A), several encode transcription factors associated with skin, colorectal, breast, and lymphoma cancers, notably IFN alpha-inducible protein 27-like-1 (IFI27L1, FC=68.0 (68)), AXIN2 (FC=2.2 (69)), lymphoid enhancer-binding factor-1 (LEF1, FC=2.0 (70)), Meis homeobox-1 (MEIS1, FC=2.4 (71)), and four-and-a-half LIM domains-1 (FHL1, FC=2.1 (72)). Interestingly, increased expression of retinoid X receptor-gamma (RXRG, FC=5.2), which is associated with sensation seeking (73), a behavioral trait common among alcoholics, was detected. Another overexpressed gene was serum deprivation protein response (SDPR, FC=2.8), which induces deformation of plasma membrane invaginations impairing endocytosis and potentially antigen presentation (74). Finally, expression of regulator of G-protein signaling-18 (RGS18, FC=3.3), growth factor-independent 1B transcription repressor

(GFI1B, FC=2.3), and rho guanine nucleotide exchange factor-4 (ARHGEF4, FC=6.1), which play a role in megakaryocyte differentiation (75, 76), were also increased.

Of the 97 genes upregulated in H7 compared to only C7, 26 mapped to the following GO terms: response to wounding, regulation of body fluids, platelet activation, wound healing, and platelet degranulation. Genes in "response to wounding" (Fig. 5B) include connexin-43 (GJA1, FC=11.0), a gap junction associated with impaired wound healing (77); IL-17F (FC=6.7), which can delay wound closure (78); and P-selectin (SELP, FC=2.3), a glycoprotein highly expressed in wounds (79). Genes with roles in cardiovascular disease mapped to the disease category "infarction" including carbonic anhydrase III (CA3, FC=5.2), glycoprotein VI (GP6, FC=2.9), phosphodiesterase-6H (PDE6H, FC=2.6), and integrin-alpha-2b (ITGA2B, FC=2.6 (80–83)). Furthermore, heavy ethanol consumption upregulated genes associated with cancer, notably transient receptor potential cation channel-M1 (TRPM1, FC=14.9), tripartite motif containing-31 (TRIM31, FC=9.8), RGS6 (FC=7.6), and CLDN5 (FC=2.2 (84–86)).

Twenty-one of the 31 genes upregulated in H7 compared to only M7 mapped to "neuro-ectodermal tumors" (Fig. 5C). These genes are either expressed at high levels in cancer including delta-like-1 (DLK1, FC=7.3 (87)) and insulin receptor substrate-1 (IRS1, FC=4.8 (88)); or involved in progression of cancer such as phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor-2 (PREX2, FC=11.7 (89)), latent transforming growth factor-beta-binding protein-2/3 (LTBP2/3, FC=3.6/2.6 (90, 91)), and stromal antigen-3 (STAG3, FC=2.3 (92)).

Moderate drinking activates genes associated with immunity and represses genes associated with cancer compared to controls

Of the 29 annotated genes upregulated in M7 compared to C7 (Fig. 6A), four play a role in chemotaxis: CXCL3 (FC=50.3, critical for leukocyte chemotaxis), IL-1α (FC=23.4, recruitment of neutrophils), CCL3 (FC=17.1, recruits T-cells (93)), and CCL4L1/2 (FC=8.1/5.5, trafficking of NK cells (94)). Other genes significantly upregulated in M7 include acute phase protein pentraxin-3 (PTX3, FC=15.3 (95)); ceruloplasmin (CP, FC=3.7) and granzyme A (GZMA, FC=2.2), expressed primarily by NK cells and play a role in host defense (96, 97); and GJA1 (FC=9.6), important for barrier function (98).

Several of the 18 genes downregulated in M7 compared to C7 (Fig. 6B) are involved in cancer progression including transmembrane protein-98 (TMEM98, FC=286.9), serine-protease temperature requirement-A1 (HTRA1, FC=24.1), DNA nucleotidylexotransferase (DNTT, FC=5.7), MMP9 (FC=3.5), and ten-eleven translocation-1 (TET1, FC=3.0 (99–103)). Interestingly, ABCA9 (FC=6.9), retinoic acid-binding receptor-related orphan receptor-C (RORC, FC=3.6), 2′-5′-oligoadenylate synthetase-2 (OAS2, FC=2.6), CD84 (FC=2.2), and myxovirus resistance protein-1 (MX1, FC=2.2), which play a role in innate immunity, were downregulated in M7.

Heavy ethanol consumption alters the expression of microRNAs involved in cancer and immune function

To begin uncovering the mechanisms underlying changes in gene expression regulation with moderate and heavy ethanol consumption, we compared the miRNA expression profiles of the same PBMCs isolated from controls, moderate, and heavy drinkers on day 7 post-MVA vaccination. MiRNAs are ~22 nucleotide long endogenous RNAs that target mRNAs for translational repression or degradation (104), and several reports indicate that ethanol can modulate miRNA expression (105). As described for mRNA expression, the largest differences in miRNA expression were observed between controls and heavy drinkers, and only a few miRNAs were differentially expressed between controls and moderate drinkers. Interestingly, no differentially expressed miRNAs were detected between heavy and moderate drinkers. There were 79 differentially expressed miRNAs between controls and heavy drinkers, 37 of which were upregulated (Fig. 7A) and 42 were downregulated (Fig. 7B).

Importantly, 53 of these miRNAs have mRNA targets within our dataset. Heavy drinking led to the upregulation of 29 microRNAs known to regulate 25 target mRNAs that were downregulated in our dataset. A subset of these mRNA-miRNA pairs are shown in Fig. 7C; for a complete list, please refer to Table 1. Some of the downregulated mRNAs were targeted by several differentially expressed miRNAs. For instance, miR-16, miR-15b, miR-195, and miR-374b target VEGFA, which was downregulated >5-fold. Similarly, miR-30b and miR-30c target SH2B3, downregulated 2 fold, while miR-125a and miR-125b both target SCARB1, which was downregulated >3-fold. Of the 24 microRNAs downregulated with heavy drinking, 7 were associated with an increase in their mRNA targets (Fig. 7C, Table 1). For example, the downregulated miR-101 targets GJA1, which was upregulated 11-fold with heavy drinking. MiR-144 targets AXIN2, which was upregulated 2-fold. MiR-183 and miR-202 both target ROBO2, which was upregulated more than 3-fold. Finally, miR-29a, miR-29b, and miR-29c all target SH3PXD2A, which was upregulated 4-fold in our dataset.

DISCUSSION

Using a macaque model of ethanol self-administration, we recently showed that heavy ethanol consumption suppresses, whereas moderate ethanol consumption enhances, T and B-cell responses to MVA (16). The goal of this study was to uncover the molecular mechanisms underlying this dose-dependent effect. We used RNA-Seq to identify changes in gene expression on day 7 following vaccination with MVA, the earliest time point at which we detected differences in antibody and T-cell responses (16). We also sequenced small RNA molecules to gain insight into mechanisms underlying the changes in gene regulation. Overall, our study revealed robust changes in gene and miRNA expression between control, moderate, and heavy drinkers, with fewer changes between moderate drinkers and controls compared to either group versus heavy drinking status.

The changes in gene expression reported herein provide novel insight into the reduced immune response to vaccination and the increased vulnerability to infection seen in humans with AUD. Specifically, we detected large decreases in the expression of microbial sensors

that are critical in the detection of bacterial peptides (FPR2/3 (106)), LPS (MD2, CD14, TLR4), bacterial flagellin (TLR5), and certain helminths and filoviruses (CLEC10A (49)). There was also decreased expression of genes important for antigen presentation (ABCC2/3, CLEC10A (49, 50)); recruitment of immune cells (C3AR1, C5AR1, CCR2, CXCL3, CXCL8, CXCL10, CCL3, CCL4L1/2, ICAM1 (31, 93, 107–109)); and soluble mediators that play a role in response to infection (TNFα, TNFRSF1A, IFNγ, IFNGR1, IL-1R, IL-1R1, IL-1RL1, IL-1β, S100A8/9, LL37, EPX, GRZMA (46, 110)). As previously described (111), expression of interferon signaling interferon receptor-2 (IFNAR2) was downregulated in heavy drinkers, which would contribute to deficits in both innate and adaptive immunity.

We also found decreased expression of lymphocyte activation markers including CD83 and KLRG1 (55). In our previous study, excessive ethanol consumption suppressed whereas moderate ethanol consumption enhanced MVA-specific IgG responses (17). These defects in antibody production could be partially explained by the decreased expression of transcription factors BCL3 and BCL6, which are important in germinal center formation, isotype class switching, and hypermutation (61, 62), SH2B3, which regulates B-cell development (54), and SAMSN1, an adapter protein involved in immune cell signaling (57). Reduced expression of KLF10, which suppresses regulatory T-cells by upregulating TGFβ (63), could further explain the reduction in T and B-cell responses.

Our study also revealed insight into the mechanisms by which moderate alcohol consumption stimulates immunity. Animals that drank moderate amounts of ethanol showed increased expression of chemokines CCL3 and CCL4L1, which signal through CCR5 to recruit memory T-cells (93), and IL-1α and CXCL3, which recruit neutrophils. Although a vigorous innate immune response is critical, it is equally important for the host to minimize damaging inflammation. PTX3, which plays a role in the resolution of inflammation (95), and the NFκB inhibitor, NFKBIA, were significantly upregulated with moderate drinking. These observations are in line with previous studies that showed that moderate alcohol consumption in humans significantly alters genes involved in B-cell, T-cell, and IL-15 signaling pathways, and attenuates NFκB signaling pathways in leukocytes (112).

Several of the genes that were differentially expressed with ethanol consumption have previously been described as being important for mounting immune responses to vaccination. For instance, studies that investigated yellow fever vaccine (YF-17D)-induced signatures in blood of healthy adults have reported significant increases in the expression of proinflammatory mediators CXCL10 and IL-1α and complement gene C3AR1, which were found to be predictive of robust vaccine responses (113). Another study identified ETS2 as an additional key regulator of the early innate immune response to YF-17D (114). In our study, we observed a 17-fold downregulation of CXCL10, a 3-fold downregulation of C3AR1, and a 2.5-fold downregulation of ETS2 with excessive ethanol consumption, which could explain the suppression of vaccine responses in this cohort. In contrast, we detected a 23-fold upregulation of IL-1α in moderate drinkers compared to controls, re-enforcing the association of this marker with successful immune responses.

Our gene expression analysis is also in line with clinical observations linking alcohol abuse with impaired wound healing (115), increased susceptibility of wound infections (116), and delay of wound closure (117). This defect has significant clinical ramifications since half the emergency room trauma cases involve alcohol exposure (118). Previous studies showed that ethanol exposure at the time of traumatic injury impairs wound closure via decreased pro-inflammatory cytokine release, neutrophil recruitment, and phagocytic function (115). Our gene expression data support and extend these earlier observations. We detected significantly decreased expression of multiple components of the innate immune system that play a critical role in the prevention of wound infections including pattern recognition receptors (FPR2/3, NALP3, NOD2, MD2, CD14, TLR4, TLR5, TLR8, CLEC10A, SCARB1), proinflammatory cytokines and their receptors (TNFRSF1A, IFNγ, IFNgγ1, IL-1R, IL-1R1, IL-1RL1, IL-1β, IL13RA1), as well as chemokines and their receptors (CXCL2, CXCL8, CXCL10, CCR1, CCR2, OSM, CSF3R, CSF1).

Earlier studies suggested the disruption of VEGF signaling and reduced expression of HIF-1α in endothelial cells with chronic alcohol consumption interferes with wound closure (117). Our gene expression data also show a significant decrease in the expression of both of those genes with excessive ethanol consumption. Moreover, we detected fewer transcripts of NRP1, which is expressed by endothelial cells and associates with the VEGF receptor to promote angiogenesis and wound repair (119). We also report decreased expression of CELSR1 and SDC2 that have been shown to promote effective wound repair (27, 120) as well as secreted proteins such as fibronectin and MMP-1 which promote platelet aggregation, angiogenesis, and tissue remodeling (121, 122). The expression of additional growth factors, HGF and HB-EGF, which promote angiogenesis and tissue regeneration, was also significantly decreased (28). Furthermore, heavy alcohol abuse increased expression of additional genes known to interfere with wound healing (PSEL, VWF, CX43, IL-17 (78, 79, 123)).

Additionally, our data is in line with clinical observations that heavy alcohol consumption is associated with increased incidence of chronic obstructive pulmonary disease (COPD (124, 125)), lung injury in response to inflammatory insults (126), acute respiratory distress syndrome (ARDS (127)), and risk of mortality in acute lung injury patients (128). Impaired immunity and increased oxidative stress, both consequences of AUD, are considered risk factors for COPD and ARDS (129). Our transcriptome analysis provides new insight into the mechanisms that underlie increased susceptibility and severity of lung injury and chronic lung inflammatory diseases. Heavy ethanol consumption was associated with downregulation of several genes important for maintaining lung homeostasis that can be categorized into: transcription factors (AHR, P21, RORg, ATF/CREB); receptors (TREM1, FCgR, NOR1); immune signaling molecules (IL-1R, IL-1β, CXCL8, CD14, CD163, G-CSF, TNFR1, TLR5, ALOX15, ADAM12); transporters (SLC11A1/16A1); and growth factors (VEGF, HBEGF, HGF). AHR and RORg play a role in suppressing lung inflammation (35, 130) while decreased p21 expression is associated with hypoxia-induced lung disease (131). In addition, transcripts associated with immune genes that promote host defense against pulmonary infection (ALOX15, CD14, G-CSF, TREM1, TLR5, NRAMP1, TNFR1) were reduced with heavy drinking (132–135). Critical growth factors important for repairing lung injury were also downregulated. Decreased levels of VEGF correlate with loss of alveolar

structure in emphysema patients (34) and a compromised integrity of the alveolar-capillary barrier (136). Finally, HGF is important for lung development and also promotes regeneration after lung injury in animals (28).

Chronic alcohol consumption is associated with an increased risk of cardiovascular disease (137) and stroke (138). Our analysis has revealed increased expression of genes implicated in heart disease including CLDN5, VWF, PDE6H, ITGA2B, and CA3 (80, 82, 83, 139, 140), as well as megakaryocyte differentiation (RGS18, GFI1B, and ARHGEF4 (75, 76)). ITGA2B is used as a biomarker for myocardial infarction risk and therapeutic modulation of phosphodiesterases is one strategy for treating cardiovascular disease. Interestingly, claudin-5 levels are reduced in human and mice models of cardiomyopathy; therefore, its increased expression here might be a compensatory mechanism.

Finally, heavy alcohol use is a major risk factor for liver, head and neck, and colorectal cancers (141–143). Our gene expression analysis showed increased expression of several genes that promote cancer progression with chronic heavy alcohol consumption (LTBP2/3, IRS-1, SFRP5, LEF1, DLK1, STAG3, and H2B). Higher levels of IRS1are found in hepatocellular carcinoma, breast, ovarian, and colorectal cancers (144–147). Increased expression of LEF1 is associated with human endometrial tumors, prostate, and colon cancer (148–150). DLK1 is also expressed at higher levels in colon adenocarcinomas, pancreatic islet carcinomas, and small cell lung carcinomas (87). In contrast, moderate alcohol consumption is associated with a reduced risk of developing kidney cancer (151), Hodgkin’s lymphoma (152), and thyroid cancer (153). Our study revealed moderate drinking repressed genes associated with reduced cancer incidence including TMEM98 (102), HTRA1 (101), DNTT (99), TET1 (100), and MMP9 (103).

We also investigated differences in miRNA expression levels between the three experimental groups. MiRNAs can modulate gene expression through translational repression or degradation of target mRNAs and play a critical role in regulating immune function (154). We identified several differentially expressed miRNAs with validated mRNA targets present in our RNA-Seq dataset. Interestingly, and as described for mRNA, several of these upregulated miRNAs have also been implicated in the development and progression of cancer. For instance, miR-494 has been shown to be upregulated in hepatocellular carcinomas and promote proliferation in tumor cells (155), while miR-106a is upregulated in gastric, colorectal, and pancreatic cancers in humans (156). As described previously in human hepatocytes and cholangiocytes treated with ethanol (157), miR-34a was upregulated greater than 5-fold in our dataset. Furthermore, ethanol-induced hypomethylation of the miR-34a promoter, which results in increased expression of this miRNA, plays a role in the development of alcoholic liver disease (157).

Moreover, several upregulated miRNAs in our dataset are involved in modulating immune responses. For example, and as we recently reported (17), miR-221 was upregulated in PBMCs of heavy drinkers. We previously showed that increased levels of miR-221 resulted in decreased expression of transcription factors STAT3 and ARNT, which in turn regulate expression of VEGF, G-CSF, and HGF (17, 158). Indeed, VEGFA and HGF were downregulated in this study by 5- and 2-fold, respectively. Another target of miR-221,

RGS6, was also downregulated in this study. In addition, upregulation of miR-125b interferes with the innate immune response following LPS stimulation or microbial infection (159). Finally, miR-223, up-regulated 19-fold in our dataset, inhibits NFκB activation, angiogenesis, and endothelial cell proliferation, thereby impairing wound healing (160) and inflammation (161).

Many of the downregulated miRNAs are also involved in cancer. For instance, miR-203 has been identified as a tumor suppressor and inhibits proliferation in colorectal cancer cell lines (162). Similarly, expression of miR-144 is significantly decreased in human lung cancer and inhibits proliferation in lung cancer cell lines (163). Finally, chronic ethanol feeding in a mouse model of alcoholic steatohepatitis has also led to downregulation of miR-183 (164). Additionally, most of the downregulated miRNAs modulate immunity. MiR-183 levels have been shown to be positively associated with phagocytosis by macrophages (165). As we reported previously (17, 158), miR-29a expression was modulated by heavy drinking. Specifically, miR29a was downregulated and its target SH3PXD2A was upregulated. Finally, in addition to its role as a tumor suppressor, increased expression of miR-203 has also been shown to be important for anti-inflammatory responses (166).

In summary, our studies revealed that heavy ethanol consumption results in the downregulation of genes that promote resolution of infection, wound healing, and protect against obstructive lung diseases and cancer whereas, moderate drinkers showed increased expression of genes associated with enhancing immune responses. Heavy drinking status also resulted in upregulation of genes involved in impaired wound healing and cancer progression compared to controls and moderate drinkers, whereas moderate ethanol consumption lowered expression of genes associated with cancer. Moreover, heavy ethanol consumption altered the expression of several miRNAs whose targets were differentially expressed in our data set and are involved in cancer progression and immune function. One of the strengths of this study is that we used an outbred animal model of voluntary self-administration that faithfully recapitulates human behavior and physiology. However, a caveat of the current study is that only 3 animals per group were analyzed. Future studies are needed to extend these observations using a larger cohort of animals to other infectious agents such as influenza. Future studies will also investigate the mechanisms underlying dose-dependent changes in gene expression by uncovering factors regulating gene expression such as epigenetic changes within specific immune cells that an influence expression of both mRNA and miRNA. Using the nonhuman primate model of alcohol self-administration, longitudinal gene regulation, and epigenetic changes in immune cells and target organs, offer the promise for understanding the complicated and dose-dependent impact of alcohol on immunity and health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Sumana Pasala for help with RNA extraction and library construction.

This work was supported by NIAAA grant AA021947-02, U01 AA013510, R24 AA109431, and NSF grant ABI-0957099. Sequencing was carried out by the UCR Genomics core supported by NIH 1S10RR028934-01.

Abbreviations used in this article

MVA	Modified Vaccinia Ankara
BEC	blood ethanol concentration
DEG	differentially expressed gene
miRNA	microRNA

References

1. Bhattacharya R, Shuhart MC. Hepatitis C and alcohol: interactions, outcomes, and implications. J Clin Gastroenterol. 2003; 36:242–252. [PubMed: 12590237]
2. Happel KI, Nelson S. Alcohol, immunosuppression, and the lung. Proc Am Thorac Soc. 2005; 2:428–432. [PubMed: 16322595]
3. Nelson S, Bagby GJ. Alcohol and HIV Infection. Transactions of the American Clinical and Climatological Association. 2011; 122:244–253. [PubMed: 21686230]
4. Gurung P, Young BM, Coleman RA, Wiechert S, Turner LE, Ray NB, Waldschmidt TJ, Legge KL, Cook RT. Chronic ethanol induces inhibition of antigen-specific CD8+ but not CD4+ immunodominant T cell responses following Listeria monocytogenes inoculation. J Leukoc Biol. 2009; 85:34–43. [PubMed: 18820175]
5. Mason CM, Dobard E, Zhang P, Nelson S. Alcohol exacerbates murine pulmonary tuberculosis. Infect Immun. 2004; 72:2556–2563. [PubMed: 15102763]
6. Meyerholz DK, Edsen-Moore M, McGill J, Coleman RA, Cook RT, Legge KL. Chronic alcohol consumption increases the severity of murine influenza virus infections. J Immunol. 2008; 181:641–648. [PubMed: 18566431]
7. Poonia B, Nelson S, Bagby GJ, Zhang P, Quniton L, Veazey RS. Chronic alcohol consumption results in higher simian immunodeficiency virus replication in mucosally inoculated rhesus macaques. AIDS Res Hum Retroviruses. 2006; 22:589–594. [PubMed: 16796534]
8. Curtis BJ, Zahs A, Kovacs EJ. Epigenetic targets for reversing immune defects caused by alcohol exposure. Alcohol research: current reviews. 2013; 35:97–113. [PubMed: 24313169]
9. Bode C, Bode JC. Activation of the innate immune system and alcoholic liver disease: effects of ethanol per se or enhanced intestinal translocation of bacterial toxins induced by ethanol? Alcoholism, clinical and experimental research. 2005; 29:166S–171S.
10. Happel K, Nelson S. Alcohol, immunosuppression, and the lung. Proceedings of the American Thoracic Society. 2005; 2:428–460. [PubMed: 16322595]
11. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcoholism, clinical and experimental research. 2009; 33:220–232.
12. Takkouche B, Regueira-Mendez C, Garcia-Closas R, Figueiras A, Gestal-Otero JJ, Hernan MA. Intake of wine, beer, and spirits and the risk of clinical common cold. Am J Epidemiol. 2002; 155:853–858. [PubMed: 11978590]
13. Cohen S, Tyrrell DA, Russell MA, Jarvis MJ, Smith AP. Smoking, alcohol consumption, and susceptibility to the common cold. Am J Public Health. 1993; 83:1277–1283. [PubMed: 8363004]
14. Ouchi E, Niu K, Kobayashi Y, Guan L, Momma H, Guo H, Chujo M, Otomo A, Cui Y, Nagatomi R. Frequent alcohol drinking is associated with lower prevalence of self-reported common cold: a retrospective study. BMC public health. 2012; 12:987. [PubMed: 23158193]
15. Mendenhall CL, Theus SA, Roselle GA, Grossman CJ, Rouster SD. Biphasic in vivo immune function after low- versus high-dose alcohol consumption. Alcohol. 1997; 14:255–260. [PubMed: 9160803]

16. Grant KA, Leng X, Green HL, Szeliga KT, Rogers LS, Gonzales SW. Drinking typography established by scheduled induction predicts chronic heavy drinking in a monkey model of ethanol self-administration. Alcoholism, clinical and experimental research. 2008; 32:1824–1838.
17. Messaoudi I, Asquith M, Engelmann F, Park B, Brown M, Rau A, Shaw J, Grant KA. Moderate alcohol consumption enhances vaccine-induced responses in rhesus macaques. Vaccine. 2013; 32:54–61. [PubMed: 24200973]
18. Baker EJ, Farro J, Gonzales S, Helms C, Grant KA. Chronic alcohol self-administration in monkeys shows long-term quantity/frequency categorical stability. Alcoholism, clinical and experimental research. 2014; 38:2835–2843.
19. Huber W V, Carey J, Gentleman R, Anders S, Carlson M, Carvalho BS, Bravo HC, Davis S, Gatto L, Girke T, Gottardo R, Hahne F, Hansen KD, Irizarry RA, Lawrence M, Love MI, MacDonald J, Obenchain V, Oles AK, Pages H, Reyes A, Shannon P, Smyth GK, Tenenbaum D, Waldron L, Morgan M. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods. 2015; 12:115–121. [PubMed: 25633503]
20. Girke T. systemPipeR: NGS workflow and report generation environment. 2015 R package version 1.2.4 ed.
21. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012; 9:357–359. [PubMed: 22388286]
22. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology. 2013; 14:R36. [PubMed: 23618408]
23. Cunningham F, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Giron CG, Gordon L, Hourlier T, Hunt SE, Janacek SH, Johnson N, Juettemann T, Kahari AK, Keenan S, Martin FJ, Maurel T, McLaren W, Murphy DN, Nag R, Overduin B, Parker A, Patricio M, Perry E, Pignatelli M, Riat HS, Sheppard D, Taylor K, Thormann A, Vullo A, Wilder SP, Zadissa A, Aken BL, Birney E, Harrow J, Kinsella R, Muffato M, Ruffier M, Searle SM, Spudich G, Trevanion SJ, Yates A, Zerbino DR, Flicek P. Ensembl 2015. Nucleic acids research. 2015; 43:D662–669. [PubMed: 25352552]
24. Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan MT, Carey VJ. Software for computing and annotating genomic ranges. PLoS computational biology. 2013; 9:e1003118. [PubMed: 23950696]
25. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010; 26:139–140. [PubMed: 19910308]
26. Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD. Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nat Protoc. 2013; 8:1765–1786. [PubMed: 23975260]
27. Alexopoulou AN, Multhaupt HA, Couchman JR. Syndecans in wound healing, inflammation and vascular biology. Int J Biochem Cell Biol. 2007; 39:505–528. [PubMed: 17097330]
28. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003; 83:835–870. [PubMed: 12843410]
29. Campbell L, Williams H, Crompton RA, Cruickshank SM, Hardman MJ. Nod2 deficiency impairs inflammatory and epithelial aspects of the cutaneous wound-healing response. J Pathol. 2013; 229:121–131. [PubMed: 22951952]
30. Thomas PG, Dash P, Aldridge JR Jr, Ellebedy AH, Reynolds C, Funk AJ, Martin WJ, Lamkanfi M, Webby RJ, Boyd KL, Doherty PC, Kanneganti TD. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. Immunity. 2009; 30:566–575. [PubMed: 19362023]
31. DiScipio RG, Daffern PJ, Jagels MA, Broide DH, Sriramarao P. A comparison of C3a and C5a-mediated stable adhesion of rolling eosinophils in postcapillary venules and transendothelial migration in vitro and in vivo. J Immunol. 1999; 162:1127–1136. [PubMed: 9916743]
32. Schroder WA, Major L, Suhrbier A. The role of SerpinB2 in immunity. Crit Rev Immunol. 2011; 31:15–30. [PubMed: 21395508]

33. Herrera I, Cisneros J, Maldonado M, Ramirez R, Ortiz-Quintero B, Anso E, Chandel NS, Selman M, Pardo A. Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. J Biol Chem. 2013; 288:25964–25975. [PubMed: 23902766]
34. Kasahara Y, Tuder RM, Cool CD, Lynch DA, Flores SC, Voelkel NF. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. American journal of respiratory and critical care medicine. 2001; 163:737–744. [PubMed: 11254533]
35. Rico de Souza A, Zago M, Pollock SJ, Sime PJ, Phipps RP, Baglole CJ. Genetic ablation of the aryl hydrocarbon receptor causes cigarette smoke-induced mitochondrial dysfunction and apoptosis. J Biol Chem. 2011; 286:43214–43228. [PubMed: 21984831]
36. Wang CY, Lin CF. Annexin A2: its molecular regulation and cellular expression in cancer development. Disease markers. 2014; 2014:308976. [PubMed: 24591759]
37. Kuramochi H, Nakajima G, Kaneko Y, Nakamura A, Inoue Y, Yamamoto M, Hayashi K. Amphiregulin and Epiregulin mRNA expression in primary colorectal cancer and corresponding liver metastases. BMC Cancer. 2012; 12:88. [PubMed: 22409860]
38. Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature. 2001; 410:1103–1107. [PubMed: 11323674]
39. Radulescu A, Yu X, Orvets ND, Chen Y, Zhang HY, Besner GE. Deletion of the heparin-binding epidermal growth factor-like growth factor gene increases susceptibility to necrotizing enterocolitis. J Pediatr Surg. 2010; 45:729–734. [PubMed: 20385279]
40. Wang ZQ, Xing WM, Fan HH, Wang KS, Zhang HK, Wang QW, Qi J, Yang HM, Yang J, Ren YN, Cui SJ, Zhang X, Liu F, Lin DH, Wang WH, Hoffmann MK, Han ZG. The novel lipopolysaccharide-binding protein CRISPLD2 is a critical serum protein to regulate endotoxin function. J Immunol. 2009; 183:6646–6656. [PubMed: 19864597]
41. Abramovici H, Mojtabaie P, Parks RJ, Zhong XP, Koretzky GA, Topham MK, Gee SH. Diacylglycerol kinase zeta regulates actin cytoskeleton reorganization through dissociation of Rac1 from RhoGDI. Mol Biol Cell. 2009; 20:2049–2059. [PubMed: 19211846]
42. Xiang Y, Tan YR, Zhang JS, Qin XQ, Hu BB, Wang Y, Qu F, Liu HJ. Wound repair and proliferation of bronchial epithelial cells regulated by CTNNAL1. J Cell Biochem. 2008; 103:920–930. [PubMed: 17647259]
43. Gill SE, Parks WC. Metalloproteinases and their inhibitors: regulators of wound healing. Int J Biochem Cell Biol. 2008; 40:1334–1347. [PubMed: 18083622]
44. Thuraisingam T, Sam H, Moisan J, Zhang Y, Ding A, Radzioch D. Delayed cutaneous wound healing in mice lacking solute carrier 11a1 (formerly Nramp1): correlation with decreased expression of secretory leukocyte protease inhibitor. The Journal of investigative dermatology. 2006; 126:890–901. [PubMed: 16470178]
45. Okuno Y, Nakamura-Ishizu A, Kishi K, Suda T, Kubota Y. Bone marrow-derived cells serve as proangiogenic macrophages but not endothelial cells in wound healing. Blood. 2011; 117:5264–5272. [PubMed: 21411758]
46. Malik A, Batra JK. Antimicrobial activity of human eosinophil granule proteins: involvement in host defence against pathogens. Crit Rev Microbiol. 2012; 38:168–181. [PubMed: 22239733]
47. Li J, Fu Y, Zhao B, Xiao Y, Chen R. Myeloperoxidase G463A polymorphism and risk of lung cancer. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014; 35:821–829. [PubMed: 23979979]
48. Albrechtsen R, Kveiborg M, Stautz D, Vikesa J, Noer JB, Kotzsh A, Nielsen FC, Wewer UM, Frohlich C. ADAM12 redistributes and activates MMP-14, resulting in gelatin degradation, reduced apoptosis and increased tumor growth. J Cell Sci. 2013; 126:4707–4720. [PubMed: 24006261]
49. van Vliet SJ, Saeland E, van Kooyk Y. Sweet preferences of MGL: carbohydrate specificity and function. Trends Immunol. 2008; 29:83–90. [PubMed: 18249034]
50. Procko E, Gaudet R. Antigen processing and presentation: TAPping into ABC transporters. Curr Opin Immunol. 2009; 21:84–91. [PubMed: 19261456]

51. Legrand F, Tomasevic N, Simakova O, Lee CC, Wang Z, Raffeld M, Makiya MA, Palath V, Leung J, Baer M, Yarranton G, Maric I, Bebbington C, Klion AD. The eosinophil surface receptor epidermal growth factor-like module containing mucin-like hormone receptor 1 (EMR1): a novel therapeutic target for eosinophilic disorders. J Allergy Clin Immunol. 2014; 133:1439–1447. 1447 e1431–1438. [PubMed: 24530099]
52. Zhao H, Yang W, Qiu R, Li J, Xin Q, Wang X, Feng Y, Shan S, Liu Y, Gong Y, Liu Q. An intronic variant associated with systemic lupus erythematosus changes the binding affinity of Yinyang1 to downregulate WDFY4. Genes Immun. 2012; 13:536–542. [PubMed: 22972472]
53. Perry RT, Gearhart DA, Wiener HW, Harrell LE, Barton JC, Kutlar A, Kutlar F, Ozcan O, Go RC, Hill WD. Hemoglobin binding to A beta and HBG2 SNP association suggest a role in Alzheimer's disease. Neurobiol Aging. 2008; 29:185–193. [PubMed: 17157413]
54. Takaki S, Morita H, Tezuka Y, Takatsu K. Enhanced hematopoiesis by hematopoietic progenitor cells lacking intracellular adaptor protein, Lnk. J Exp Med. 2002; 195:151–160. [PubMed: 11805142]
55. Voehringer D, Koschella M, Pircher H. Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1). Blood. 2002; 100:3698–3702. [PubMed: 12393723]
56. Murray CM, Hutchinson R, Bantick JR, Belfield GP, Benjamin AD, Brazma D, Bundick RV, Cook ID, Craggs RI, Edwards S, Evans LR, Harrison R, Holness E, Jackson AP, Jackson CG, Kingston LP, Perry MW, Ross AR, Rugman PA, Sidhu SS, Sullivan M, Taylor-Fishwick DA, Walker PC, Whitehead YM, Wilkinson DJ, Wright A, Donald DK. Monocarboxylate transporter MCT1 is a target for immunosuppression. Nature chemical biology. 2005; 1:371–376.
57. Zhu YX, Benn S, Li ZH, Wei E, Masih-Khan E, Trieu Y, Bali M, McGlade CJ, Claudio JO, Stewart AK. The SH3-SAM adaptor HACS1 is up-regulated in B cell activation signaling cascades. J Exp Med. 2004; 200:737–747. [PubMed: 15381729]
58. Prazma CM, Yazawa N, Fujimoto Y, Fujimoto M, Tedder TF. CD83 expression is a sensitive marker of activation required for B cell and CD4+ T cell longevity in vivo. J Immunol. 2007; 179:4550–4562. [PubMed: 17878352]
59. Metzger ML, Michelfelder I, Goldacker S, Melkaoui K, Litzman J, Guzman D, Grimbacher B, Salzer U. Low ficolin-2 levels in common variable immunodeficiency patients with bronchiectasis. Clin Exp Immunol. 2015; 179:256–264. [PubMed: 25251245]
60. Vogler M. BCL2A1: the underdog in the BCL2 family. Cell Death Differ. 2012; 19:67–74. [PubMed: 22075983]
61. Franzoso G, Carlson L, Scharton-Kersten T, Shores EW, Epstein S, Grinberg A, Tran T, Shacter E, Leonardi A, Anver M, Love P, Sher A, Siebenlist U. Critical roles for the Bcl-3 oncoprotein in T cell-mediated immunity, splenic microarchitecture, and germinal center reactions. Immunity. 1997; 6:479–490. [PubMed: 9133427]
62. Fukuda T, Yoshida T, Okada S, Hatano M, Miki T, Ishibashi K, Okabe S, Koseki H, Hirosawa S, Taniguchi M, Miyasaka N, Tokuhisa T. Disruption of the Bcl6 gene results in an impaired germinal center formation. J Exp Med. 1997; 186:439–448. [PubMed: 9236196]
63. Cao Z, Wara AK, Icli B, Sun X, Packard RR, Esen F, Stapleton CJ, Subramaniam M, Kretschmer K, Apostolou I, von Boehmer H, Hansson GK, Spelsberg TC, Libby P, Feinberg MW. Kruppel-like factor KLF10 targets transforming growth factor-beta1 to regulate CD4(+)CD25(−) T cells and T regulatory cells. J Biol Chem. 2009; 284:24914–24924. [PubMed: 19602726]
64. Ali S, Hirschfeld AF, Mayer ML, Fortuno ES 3rd, Corbett N, Kaplan M, Wang S, Schneiderman J, Fjell CD, Yan J, Akhabir L, Aminuddin F, Marr N, Lacaze-Masmonteil T, Hegele RG, Becker A, Chan-Yeung M, Hancock RE, Kollmann TR, Daley D, Sandford AJ, Lavoie PM, Turvey SE. Functional genetic variation in NFKBIA and susceptibility to childhood asthma, bronchiolitis, and bronchopulmonary dysplasia. J Immunol. 2013; 190:3949–3958. [PubMed: 23487427]
65. Milkiewicz M, Uchida C, Gee E, Fudalewski T, Haas TL. Shear stress-induced Ets-1 modulates protease inhibitor expression in microvascular endothelial cells. J Cell Physiol. 2008; 217:502–510. [PubMed: 18636553]
66. Ju S, Zhu Y, Liu L, Dai S, Li C, Chen E, He Y, Zhang X, Lu B. Gadd45b and Gadd45g are important for anti-tumor immune responses. Eur J Immunol. 2009; 39:3010–3018. [PubMed: 19688743]

67. Chau CH, Clavijo CA, Deng HT, Zhang Q, Kim KJ, Qiu Y, Le AD, Ann DK. Etk/Bmx mediates expression of stress-induced adaptive genes VEGF, PAI-1, and iNOS via multiple signaling cascades in different cell systems. Am J Physiol Cell Physiol. 2005; 289:C444–454. [PubMed: 15788485]
68. Suomela S, Cao L, Bowcock A, Saarialho-Kere U. Interferon alpha-inducible protein 27 (IFI27) is upregulated in psoriatic skin and certain epithelial cancers. The Journal of investigative dermatology. 2004; 122:717–721. [PubMed: 15086558]
69. Schaal U, Grenz S, Merkel S, Rau TT, Hadjihannas MV, Kremmer E, Chudasama P, Croner RS, Behrens J, Sturzl M, Naschberger E. Expression and localization of axin 2 in colorectal carcinoma and its clinical implication. International journal of colorectal disease. 2013; 28:1469–1478. [PubMed: 23702820]
70. Walther N, Ulrich A, Vockerodt M, von Bonin F, Klapper W, Meyer K, Eberth S, Pukrop T, Spang R, Trumper L, Kube D. Aberrant lymphocyte enhancer-binding factor 1 expression is characteristic for sporadic Burkitt's lymphoma. Am J Pathol. 2013; 182:1092–1098. [PubMed: 23375451]
71. Wang QF, Li YJ, Dong JF, Li B, Kaberlein JJ, Zhang L, Arimura FE, Luo RT, Ni J, He F, Wu J, Mattison R, Zhou J, Wang CZ, Prabhakar S, Nobrega MA, Thirman MJ. Regulation of MEIS1 by distal enhancer elements in acute leukemia. Leukemia. 2014; 28:138–146. [PubMed: 24022755]
72. Ding L, Niu C, Zheng Y, Xiong Z, Liu Y, Lin J, Sun H, Huang K, Yang W, Li X, Ye Q. FHL1 interacts with oestrogen receptors and regulates breast cancer cell growth. J Cell Mol Med. 2011; 15:72–85. [PubMed: 19840196]
73. Alliey-Rodriguez N, Zhang D, Badner JA, Lahey BB, Zhang X, Dinwiddie S, Romanos B, Plenys N, Liu C, Gershon ES. Genome-wide association study of personality traits in bipolar patients. Psychiatric genetics. 2011; 21:190–194. [PubMed: 21368711]
74. Hansen CG, Bright NA, Howard G, Nichols BJ. SDPR induces membrane curvature and functions in the formation of caveolae. Nat Cell Biol. 2009; 11:807–814. [PubMed: 19525939]
75. Laurent B, Randrianarison-Huetz V, Kadri Z, Romeo PH, Porteu F, Dumenil D. Gfi-1B promoter remains associated with active chromatin marks throughout erythroid differentiation of human primary progenitor cells. Stem Cells. 2009; 27:2153–2162. [PubMed: 19522008]
76. Yowe D, Weich N, Prabhudas M, Poisson L, Errada P, Kapeller R, Yu K, Faron L, Shen M, Cleary J, Wilkie TM, Gutierrez-Ramos C, Hodge MR. RGS18 is a myeloerythroid lineage-specific regulator of G-protein-signalling molecule highly expressed in megakaryocytes. Biochem J. 2001; 359:109–118. [PubMed: 11563974]
77. Mori R, Power KT, Wang CM, Martin P, Becker DL. Acute downregulation of connexin43 at wound sites leads to a reduced inflammatory response, enhanced keratinocyte proliferation and wound fibroblast migration. J Cell Sci. 2006; 119:5193–5203. [PubMed: 17158921]
78. Poutahidis T, Kearney SM, Levkovich T, Qi P, Varian BJ, Lakritz JR, Ibrahim YM, Chatzigiagkos A, Alm EJ, Erdman SE. Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. PLoS One. 2013; 8:e78898. [PubMed: 24205344]
79. Dressler J, Bachmann L, Koch R, Muller E. Enhanced expression of selectins in human skin wounds. Int J Legal Med. 1999; 112:39–44. [PubMed: 9932741]
80. Liu C, Wei Y, Wang J, Pi L, Huang J, Wang P. Carbonic anhydrases III and IV autoantibodies in rheumatoid arthritis, systemic lupus erythematosus, diabetes, hypertensive renal disease, and heart failure. Clin Dev Immunol. 2012; 2012:354594. [PubMed: 23049597]
81. Yamashita Y, Naitoh K, Wada H, Ikejiri M, Mastumoto T, Ohishi K, Hosaka Y, Nishikawa M, Katayama N. Elevated plasma levels of soluble platelet glycoprotein VI (GPVI) in patients with thrombotic microangiopathy. Thrombosis research. 2014; 133:440–444. [PubMed: 24325877]
82. Knight W, Yan C. Therapeutic potential of PDE modulation in treating heart disease. Future medicinal chemistry. 2013; 5:1607–1620. [PubMed: 24047267]
83. Voora D, Cyr D, Lucas J, Chi JT, Dungan J, McCaffrey TA, Katz R, Newby LK, Kraus WE, Becker RC, Ortel TL, Ginsburg GS. Aspirin exposure reveals novel genes associated with platelet function and cardiovascular events. Journal of the American College of Cardiology. 2013; 62:1267–1276. [PubMed: 23831034]

84. Dalal MD, Morgans CW, Duvoisin RM, Gamboa EA, Jeffrey BG, Garg SJ, Chan CC, Sen HN. Diagnosis of occult melanoma using transient receptor potential melastatin 1 (TRPM1) autoantibody testing: a novel approach. Ophthalmology. 2013; 120:2560–2564. [PubMed: 24053997]

85. Sugiura T, Miyamoto K. Characterization of TRIM31, upregulated in gastric adenocarcinoma, as a novel RBCC protein. J Cell Biochem. 2008; 105:1081–1091. [PubMed: 18773414]

86. Soini Y, Eskelinen M, Juvonen P, Karja V, Haapasaari KM, Saarela A, Karihtala P. Strong claudin 5 expression is a poor prognostic sign in pancreatic adenocarcinoma. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014; 35:3803–3808. [PubMed: 24519061]

87. Yanai H, Nakamura K, Hijioka S, Kamei A, Ikari T, Ishikawa Y, Shinozaki E, Mizunuma N, Hatake K, Miyajima A. Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency. J Biochem. 2010; 148:85–92. [PubMed: 20356822]

88. Reuveni H, Flashner-Abramson E, Steiner L, Makedonski K, Song R, Shir A, Herlyn M, Bar-Eli M, Levitzki A. Therapeutic destruction of insulin receptor substrates for cancer treatment. Cancer Res. 2013; 73:4383–4394. [PubMed: 23651636]

89. Berger MF, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, Ivanova E, Watson IR, Nickerson E, Ghosh P, Zhang H, Zeid R, Ren X, Cibulskis K, Sivachenko AY, Wagle N, Sucker A, Sougnez C, Onofrio R, Ambrogio L, Auclair D, Fennell T, Carter SL, Drier Y, Stojanov P, Singer MA, Voet D, Jing R, Saksena G, Barretina J, Ramos AH, Pugh TJ, Stransky N, Parkin M, Winckler W, Mahan S, Ardlie K, Baldwin J, Wargo J, Schadendorf D, Meyerson M, Gabriel SB, Golub TR, Wagner SN, Lander ES, Getz G, Chin L, Garraway LA. Melanoma genome sequencing reveals frequent PREX2 mutations. Nature. 2012; 485:502–506. [PubMed: 22622578]

90. da Costa AN, Plymoth A, Santos-Silva D, Ortiz-Cuaran S, Camey S, Guilloreau P, Sangrajrang S, Khuhaprema T, Mendy M, Lesi OA, Chang HK, Oh JK, Lee DH, Shin HR, Kirk GD, Merle P, Beretta L, Hainaut P. Osteopontin and latent-TGF beta binding-protein 2 as potential diagnostic markers for HBV-related hepatocellular carcinoma. Int J Cancer. 2014

91. Naba A, Clauser KR, Lamar JM, Carr SA, Hynes RO. Extracellular matrix signatures of human mammary carcinoma identify novel metastasis promoters. eLife. 2014; 3:e01308. [PubMed: 24618895]

92. Notaridou M, Quaye L, Dafou D, Jones C, Song H, Hogdall E, Kjaer SK, Christensen L, Hogdall C, Blaakaer J, McGuire V, Wu AH, Van Den Berg DJ, Pike MC, Gentry-Maharaj A, Wozniak E, Sher T, Jacobs IJ, Tyrer J, Schildkraut JM, Moorman PG, Iversen ES, Jakubowska A, Medrek K, Lubinski J, Ness RB, Moysich KB, Lurie G, Wilkens LR, Carney ME, Wang-Gohrke S, Doherty JA, Rossing MA, Beckmann MW, Thiel FC, Ekici AB, Chen X, Beesley J, Gronwald J, Fasching PA, Chang-Claude J, Goodman MT, Chenevix-Trench G, Berchuck A, Pearce CL, Whittemore AS, Menon U, Pharoah PD, Gayther SA, Ramus SJ. S. Australian Ovarian Cancer Study Group/ Australian Cancer, C. Ovarian Cancer Association. Common alleles in candidate susceptibility genes associated with risk and development of epithelial ovarian cancer. Int J Cancer. 2011; 128:2063–2074. [PubMed: 20635389]

93. Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta. Science. 1993; 260:355–358. [PubMed: 7682337]

94. Morris MA, Ley K. Trafficking of natural killer cells. Curr Mol Med. 2004; 4:431–438. [PubMed: 15354873]

95. Rodriguez-Grande B, Swana M, Nguyen L, Englezou P, Maysami S, Allan SM, Rothwell NJ, Garlanda C, Denes A, Pinteaux E. The acute-phase protein PTX3 is an essential mediator of glial scar formation and resolution of brain edema after ischemic injury. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2014; 34:480–488.

96. Banha J, Marques L, Oliveira R, de Martins MF, Paixao E, Pereira D, Malho R, Penque D, Costa L. Ceruloplasmin expression by human peripheral blood lymphocytes: a new link between immunity and iron metabolism. Free Radic Biol Med. 2008; 44:483–492. [PubMed: 17991445]

97. Johnson BJ, Costelloe EO, Fitzpatrick DR, Haanen JB, Schumacher TN, Brown LE, Kelso A. Single-cell perforin and granzyme expression reveals the anatomical localization of effector CD8+ T cells in influenza virus-infected mice. Proc Natl Acad Sci U S A. 2003; 100:2657–2662. [PubMed: 12601154]

98. Djavani MM, Crasta OR, Zapata JC, Fei Z, Folkerts O, Sobral B, Swindells M, Bryant J, Davis H, Pauza CD, Lukashevich IS, Hammamieh R, Jett M, Salvato MS. Early blood profiles of virus infection in a monkey model for Lassa fever. J Virol. 2007; 81:7960–7973. [PubMed: 17522210]

99. Faber J, Kantarjian H, Roberts MW, Keating M, Freireich E, Albitar M. Terminal deoxynucleotidyl transferase-negative acute lymphoblastic leukemia. Archives of pathology & laboratory medicine. 2000; 124:92–97. [PubMed: 10629138]

100. Huang H, Jiang X, Li Z, Li Y, Song CX, He C, Sun M, Chen P, Gurbuxani S, Wang J, Hong GM, Elkahloun AG, Arnovitz S, Wang J, Szulwach K, Lin L, Street C, Wunderlich M, Dawlaty M, Neilly MB, Jaenisch R, Yang FC, Mulloy JC, Jin P, Liu PP, Rowley JD, Xu M, He C, Chen J. TET1 plays an essential oncogenic role in MLL-rearranged leukemia. Proc Natl Acad Sci U S A. 2013; 110:11994–11999. [PubMed: 23818607]

101. D'Angelo V, Pecoraro G, Indolfi P, Iannotta A, Donofrio V, Errico ME, Indolfi C, Ramaglia M, Lombardi A, Di Martino M, Gigantino V, Baldi A, Caraglia M, De Luca A, Casale F. Expression and localization of serine protease Htra1 in neuroblastoma: correlation with cellular differentiation grade. J Neurooncol. 2014; 117:287–294. [PubMed: 24493577]

102. Ng KT, Lo CM, Guo DY, Qi X, Li CX, Geng W, Liu XB, Ling CC, Ma YY, Yeung WH, Shao Y, Poon RT, Fan ST, Man K. Identification of transmembrane protein 98 as a novel chemoresistance-conferring gene in hepatocellular carcinoma. Mol Cancer Ther. 2014; 13:1285–1297. [PubMed: 24608572]

103. Lotfi A, Mohammadi G, Tavassoli A, Mousaviagdas M, Chavoshi H, Saniee L. Serum Levels of MMP9 and MMP2 in Patients with Oral Squamous Cell Carcinoma. Asian Pacific journal of cancer prevention: APJCP. 2015; 16:1327–1330. [PubMed: 25743793]

104. Tomankova T, Petrek M, Gallo J, Kriegova E. MicroRNAs: emerging regulators of immune-mediated diseases. Scandinavian journal of immunology. 2011

105. Shukla SD, Lim RW. Epigenetic effects of ethanol on the liver and gastrointestinal system. Alcohol research: current reviews. 2013; 35:47–55. [PubMed: 24313164]

106. Bufe B, Schumann T, Kappl R, Bogeski I, Kummerow C, Podgorska M, Smola S, Hoth M, Zufall F. Recognition of bacterial signal peptides by mammalian formyl peptide receptors: a new mechanism for sensing pathogens. J Biol Chem. 2015

107. Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, Mack M, Charo IF. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. J Clin Invest. 2007; 117:902–909. [PubMed: 17364026]

108. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol. 2002; 168:3195–3204. [PubMed: 11907072]

109. Howard OM, Turpin JA, Goldman R, Modi WS. Functional redundancy of the human CCL4 and CCL4L1 chemokine genes. Biochem Biophys Res Commun. 2004; 320:927–931. [PubMed: 15240137]

110. Narumi K, Miyakawa R, Ueda R, Hashimoto H, Yamamoto Y, Yoshida T, Aoki K. Proinflammatory Proteins S100A8/S100A9 Activate NK Cells via Interaction with RAGE. J Immunol. 2015; 194:5539–5548. [PubMed: 25911757]

111. Beech RD, Qu J, Leffert JJ, Lin A, Hong KA, Hansen J, Umlauf S, Mane S, Zhao H, Sinha R. Altered expression of cytokine signaling pathway genes in peripheral blood cells of alcohol dependent subjects: preliminary findings. Alcoholism, clinical and experimental research. 2012; 36:1487–1496.

112. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HF. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. The British journal of nutrition. 2011; 108:620–627. [PubMed: 22142458]

113. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, Pulendran B. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol. 2009; 10:116–125. [PubMed: 19029902]

114. Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, Filali-Mouhim A, Moser JM, Mehta RS, Drake DR 3rd, Castro E, Akondy R, Rinfret A, Yassine-Diab B, Said EA, Chouikh Y, Cameron MJ, Clum R, Kelvin D, Somogyi R, Greller LD, Balderas RS, Wilkinson P, Pantaleo G, Tartaglia J, Haddad EK, Sekaly RP. Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med. 2008; 205:3119–3131. [PubMed: 19047440]

115. Greiffenstein P, Molina PE. Alcohol-induced alterations on host defense after traumatic injury. J Trauma. 2008; 64:230–240. [PubMed: 18188126]

116. Choudhry MA I, Chaudry H. Alcohol intoxication and post-burn complications. Front Biosci. 2006; 11:998–1005. [PubMed: 16146791]

117. Radek KA, Kovacs EJ, Gallo RL, DiPietro LA. Acute ethanol exposure disrupts VEGF receptor cell signaling in endothelial cells. Am J Physiol Heart Circ Physiol. 2008; 295:H174–184. [PubMed: 18469146]

118. Cherpitel CJ. Focus on: the burden of alcohol use--trauma and emergency outcomes. Alcohol research: current reviews. 2013; 35:150–154. [PubMed: 24881323]

119. Raimondi C, Fantin A, Lampropoulou A, Denti L, Chikh A, Ruhrberg C. Imatinib inhibits VEGF-independent angiogenesis by targeting neuropilin 1-dependent ABL1 activation in endothelial cells. J Exp Med. 2014; 211:1167–1183. [PubMed: 24863063]

120. Caddy J, Wilanowski T, Darido C, Dworkin S, Ting SB, Zhao Q, Rank G, Auden A, Srivastava S, Papenfuss TA, Murdoch JN, Humbert PO, Parekh V, Boulos N, Weber T, Zuo J, Cunningham JM, Jane SM. Epidermal wound repair is regulated by the planar cell polarity signaling pathway. Dev Cell. 2010; 19:138–147. [PubMed: 20643356]

121. To WS, Midwood KS. Plasma and cellular fibronectin: distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair. 2011; 4:21. [PubMed: 21923916]

122. Gu Q, Wang D, Gao Y, Zhou J, Peng R, Cui Y, Xia G, Qing Q, Yang H, Liu J, Zhao M. Expression of MMP1 in surgical and radiation-impaired wound healing and its effects on the healing process. J Environ Pathol Toxicol Oncol. 2002; 21:71–78. [PubMed: 11934016]

123. Tokunaga F, Nakagawa T, Nakahara M, Saeki Y, Taniguchi M, Sakata S, Tanaka K, Nakano H, Iwai K. SHARPIN is a component of the NF-kappaB-activating linear ubiquitin chain assembly complex. Nature. 2011; 471:633–636. [PubMed: 21455180]

124. Hirayama F, Lee AH, Binns CW, Oga T, Nishimura K. Alcohol consumption in patients with chronic obstructive pulmonary disease in Japan. Asia Pac J Public Health. 2008; 20(Suppl):87–94. [PubMed: 19533866]

125. Tabak C, Smit HA, Rasanen L, Fidanza F, Menotti A, Nissinen A, Feskens EJ, Heederik D, Kromhout D. Alcohol consumption in relation to 20-year COPD mortality and pulmonary function in middle-aged men from three European countries. Epidemiology. 2001; 12:239–245. [PubMed: 11246587]

126. Joshi PC, Guidot DM. The alcoholic lung: epidemiology, pathophysiology, and potential therapies. Am J Physiol Lung Cell Mol Physiol. 2007; 292:L813–823. [PubMed: 17220370]

127. Prout M, Martin GS, Drexler K, Brown LA, Guidot DM. Alcohol abuse and acute lung injury: can we target therapy? Expert Rev Respir Med. 2007; 1:197–207. [PubMed: 20477184]

128. Clark BJ, Williams A, Feemster LM, Bradley KA, Macht M, Moss M, Burnham EL. Alcohol screening scores and 90-day outcomes in patients with acute lung injury. Crit Care Med. 2013; 41:1518–1525. [PubMed: 23538449]

129. Kaphalia L, Calhoun WJ. Alcoholic lung injury: metabolic, biochemical and immunological aspects. Toxicol Lett. 2013; 222:171–179. [PubMed: 23892124]

130. Tilley SL, Jaradat M, Stapleton C, Dixon D, Hua X, Erikson CJ, McCaskill JG, Chason KD, Liao G, Jania L, Koller BH, Jetten AM. Retinoid-related orphan receptor gamma controls immunoglobulin production and Th1/Th2 cytokine balance in the adaptive immune response to allergen. J Immunol. 2007; 178:3208–3218. [PubMed: 17312169]

131. Gao Y, Xue XD, Li JY, Wang N. Expression and roles of CDK4 and p21 in lung tissues of premature rats with hyperoxia-induced chronic lung disease. Zhongguo Dang Dai Er Ke Za Zhi. 2007; 9:595–600. [PubMed: 18082050]

132. Hermesh T, Moran TM, Jain D, Lopez CB. Granulocyte colony-stimulating factor protects mice during respiratory virus infections. PLoS One. 2012; 7:e37334. [PubMed: 22615983]

133. Song YS, Yang EM, Kim SH, Jin HJ, Park HS. Effect of genetic polymorphism of ALOX15 on aspirin-exacerbated respiratory disease. International archives of allergy and immunology. 2012; 159:157–161. [PubMed: 22652554]

134. Klesney-Tait J, Keck K, Li X, Gilfillan S, Otero K, Baruah S, Meyerholz DK, Varga SM, Knudson CJ, Moninger TO, Moreland J, Zabner J, Colonna M. Transepithelial migration of neutrophils into the lung requires TREM-1. J Clin Invest. 2013; 123:138–149. [PubMed: 23241959]

135. Koh WJ, Kwon OJ, Kim EJ, Lee KS, Ki CS, Kim JW. NRAMP1 gene polymorphism and susceptibility to nontuberculous mycobacterial lung diseases. Chest. 2005; 128:94–101. [PubMed: 16002921]

136. Tang K, Rossiter HB, Wagner PD, Breen EC. Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. J Appl Physiol. 2004; 97:1559–1566. 1985. discussion 1549. [PubMed: 15208295]

137. O'Keefe JH, Bhatti SK, Bajwa A, DiNicolantonio JJ, Lavie CJ. Alcohol and cardiovascular health: the dose makes the poison...or the remedy. Mayo Clin Proc. 2014; 89:382–393. [PubMed: 24582196]

138. Ikehara S, Iso H, Yamagishi K, Kokubo Y, Saito I, Yatsuya H, Inoue M, Tsugane S. Alcohol consumption and risk of stroke and coronary heart disease among Japanese women: the Japan Public Health Center-based prospective study. Prev Med. 2013; 57:505–510. [PubMed: 23859928]

139. Spiel AO, Gilbert JC, Jilma B. von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. Circulation. 2008; 117:1449–1459. [PubMed: 18347221]

140. Mays TA, Binkley PF, Lesinski A, Doshi AA, Quaile MP, Margulies KB, Janssen PM, Rafael-Fortney JA. Claudin-5 levels are reduced in human end-stage cardiomyopathy. J Mol Cell Cardiol. 2008; 45:81–87. [PubMed: 18513742]

141. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V. Carcinogenicity of alcoholic beverages. Lancet Oncol. 2007; 8:292–293. [PubMed: 17431955]

142. Grewal P V, Viswanathen A. Liver cancer and alcohol. Clinics in liver disease. 2012; 16:839–850. [PubMed: 23101985]

143. Fedirko V, Tramacere I, Bagnardi V, Rota M, Scotti L, Islami F, Negri E, Straif K, Romieu I, La Vecchia C, Boffetta P, Jenab M. Alcohol drinking and colorectal cancer risk: an overall and dose-response meta-analysis of published studies. Ann Oncol. 2011; 22:1958–1972. [PubMed: 21307158]

144. Rocha RL, Hilsenbeck SG, Jackson JG, VanDenBerg CL, Weng C, Lee AV, Yee D. Insulin-like growth factor binding protein-3 and insulin receptor substrate-1 in breast cancer: correlation with clinical parameters and disease-free survival. Clin Cancer Res. 1997; 3:103–109. [PubMed: 9815544]

145. Cantarini MC, de la Monte SM, Pang M, Tong M, D'Errico A, Trevisani F, Wands JR. Aspartyl-asparagyl beta hydroxylase over-expression in human hepatoma is linked to activation of insulin-like growth factor and notch signaling mechanisms. Hepatology (Baltimore, Md. 2006; 44:446–457.

146. Ravikumar S, Perez-Liz G, Del Vale L, Soprano DR, Soprano KJ. Insulin receptor substrate-1 is an important mediator of ovarian cancer cell growth suppression by all-trans retinoic acid. Cancer Res. 2007; 67:9266–9275. [PubMed: 17909034]

147. Esposito DL, Aru F, Lattanzio R, Morgano A, Abbondanza M, Malekzadeh R, Bishehsari F, Valanzano R, Russo A, Piantelli M, Moschetta A, Lotti LV, Mariani-Costantini R. The insulin receptor substrate 1 (IRS1) in intestinal epithelial differentiation and in colorectal cancer. PLoS One. 2012; 7:e36190. [PubMed: 22558377]

148. Shelton DN, Fornalik H, Neff T, Park SY, Bender D, DeGeest K, Liu X, Xie W, Meyerholz DK, Engelhardt JF, Goodheart MJ. The role of LEF1 in endometrial gland formation and carcinogenesis. PLoS One. 2012; 7:e40312. [PubMed: 22792274]

149. Wu L, Zhao JC, Kim J, Jin HJ, Wang CY, Yu J. ERG is a critical regulator of Wnt/LEF1 signaling in prostate cancer. Cancer Res. 2013; 73:6068–6079. [PubMed: 23913826]

150. Wang WJ, Yao Y, Jiang LL, Hu TH, Ma JQ, Liao ZJ, Yao JT, Li DF, Wang SH, Nan KJ. Knockdown of lymphoid enhancer factor 1 inhibits colon cancer progression in vitro and in vivo. PLoS One. 2013; 8:e76596. [PubMed: 24098538]

151. Lee JE, Hunter DJ, Spiegelman D, Adami HO, Albanes D, Bernstein L, van den Brandt PA, Buring JE, Cho E, Folsom AR, Freudenheim JL, Giovannucci E, Graham S, Horn-Ross PL, Leitzmann MF, McCullough ML, Miller AB, Parker AS, Rodriguez C, Rohan TE, Schatzkin A, Schouten LJ, Virtanen M, Willett WC, Wolk A, Zhang SM, Smith-Warner SA. Alcohol intake and renal cell cancer in a pooled analysis of 12 prospective studies. Journal of the National Cancer Institute. 2007; 99:801–810. [PubMed: 17505075]

152. Gorini G, Stagnaro E, Fontana V, Miligi L, Ramazzotti V, Amadori D, Rodella S, Tumino R, Crosignani P, Vindigni C, Fontana A, Vineis P, Seniori Costantini A. Alcohol consumption and risk of Hodgkin's lymphoma and multiple myeloma: a multicentre case-control study. Ann Oncol. 2007; 18:143–148. [PubMed: 17047000]

153. Allen NE, Beral V, Casabonne D, Kan SW, Reeves GK, Brown A, Green J. Moderate alcohol intake and cancer incidence in women. Journal of the National Cancer Institute. 2009; 101:296–305. [PubMed: 19244173]

154. O'Connell R, Rao D, Chaudhuri A, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nature reviews Immunology. 2010; 10:111–133.

155. Lim L, Balakrishnan A, Huskey N, Jones KD, Jodari M, Ng R, Song G, Riordan J, Anderton B, Cheung ST, Willenbring H, Dupuy A, Chen X, Brown D, Chang AN, Goga A. MicroRNA-494 within an oncogenic microRNA megacluster regulates G1/S transition in liver tumorigenesis through suppression of mutated in colorectal cancer. Hepatology (Baltimore, Md. 2014; 59:202–215.

156. Li P, Xu Q, Zhang D, Li X, Han L, Lei J, Duan W, Ma Q, Wu Z, Wang Z. Upregulated miR-106a plays an oncogenic role in pancreatic cancer. FEBS Lett. 2014; 588:705–712. [PubMed: 24444603]

157. Meng F, Glaser SS, Francis H, Yang F, Han Y, Stokes A, Staloch D, McCarra J, Liu J, Venter J, Zhao H, Liu X, Francis T, Swendsen S, Liu CG, Tsukamoto H, Alpini G. Epigenetic regulation of miR-34a expression in alcoholic liver injury. Am J Pathol. 2012; 181:804–817. [PubMed: 22841474]

158. Asquith M, Pasala S, Engelmann F, Haberthur K, Meyer C, Park B, Grant KA, Messaoudi I. Chronic Ethanol Consumption Modulates Growth Factor Release, Mucosal Cytokine Production, and MicroRNA Expression in Nonhuman Primates. Alcoholism, clinical and experimental research. 2013

159. Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, Fabbri M, Alder H, Liu CG, Calin GA, Croce CM. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol. 2007; 179:5082–5089. [PubMed: 17911593]

160. Shi L, Fisslthaler B, Zippel N, Fromel T, Hu J, Elgheznawy A, Heide H, Popp R, Fleming I. MicroRNA-223 antagonizes angiogenesis by targeting beta1 integrin and preventing growth factor signaling in endothelial cells. Circ Res. 2013; 113:1320–1330. [PubMed: 24044949]

161. Haneklaus M, Gerlic M, O'Neill LA, Masters SL. miR-223: infection, inflammation and cancer. J Intern Med. 2013; 274:215–226. [PubMed: 23772809]

162. Raisch J, Darfeuille-Michaud A, Nguyen HT. Role of microRNAs in the immune system, inflammation and cancer. World J Gastroenterol. 2013; 19:2985–2996. [PubMed: 23716978]

163. Chen S, Li P, Li J, Wang Y, Du Y, Chen X, Zang W, Wang H, Chu H, Zhao G, Zhang G. MiR-144 inhibits proliferation and induces apoptosis and autophagy in lung cancer cells by targeting TIGAR. Cell Physiol Biochem. 2015; 35:997–1007. [PubMed: 25660220]

164. Dolganiuc A, Petrasek J, Kodys K, Catalano D, Mandrekar P, Velayudham A, Szabo G. MicroRNA expression profile in Lieber-DeCarli diet-induced alcoholic and methionine choline deficient diet-induced nonalcoholic steatohepatitis models in mice. Alcoholism, clinical and experimental research. 2009; 33:1704–1710.

165. Zhang C, Wang Q, Xi X, Jiao J, Xu W, Huang J, Lai Z. High serum miR-183 level is associated with the bioactivity of macrophage derived from tuberculosis patients. International journal of clinical and experimental pathology. 2015; 8:655–659. [PubMed: 25755759]

166. Primo MN, Bak RO, Schibler B, Mikkelsen JG. Regulation of pro-inflammatory cytokines TNFalpha and IL24 by microRNA-203 in primary keratinocytes. Cytokine. 2012; 60:741–748. [PubMed: 22917968]

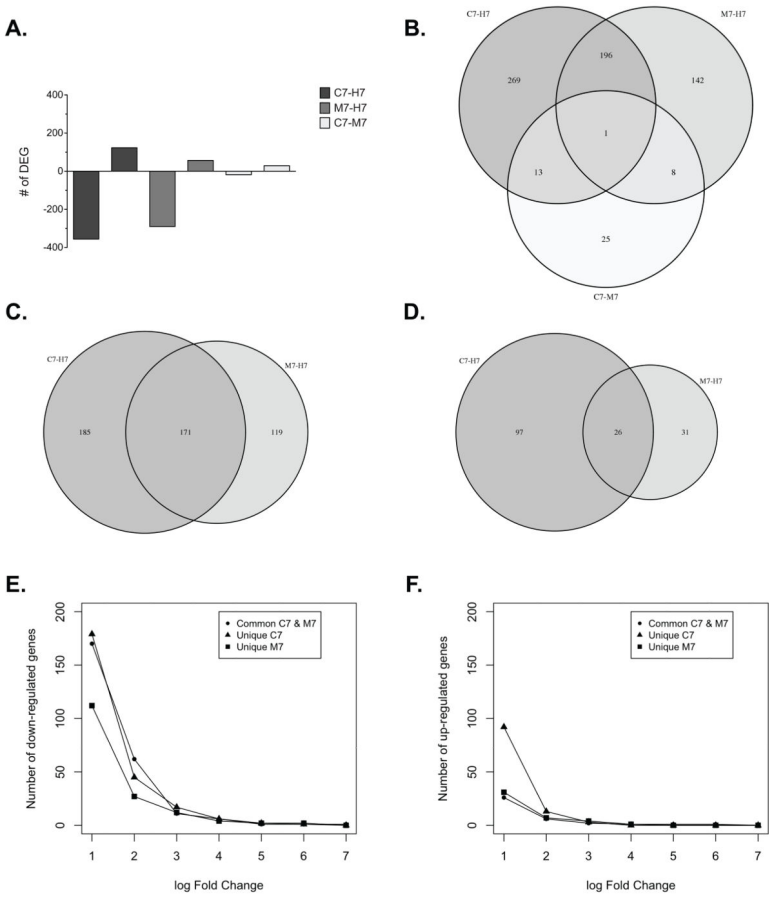

Figure 1. Peripheral blood mononuclear cell (PBMC) gene expression

(A) Bar graph showing the number of downregulated and upregulated genes for each comparison. (B) Venn diagram depicting the overlap of the annotated DEGs between controls (C), moderate drinkers (M), and heavy drinkers (H) on day 7 after booster vaccination (C7, M7 and H7 respectively). (C) Venn diagram depicting the overlap between genes that are downregulated with heavy alcohol consumption compared to controls and moderate drinkers. (D) Venn diagram depicting the overlap between genes that are upregulated with heavy alcohol consumption compared to controls and moderate drinkers. (E, F) Fold change of (E) downregulated and (F) upregulated genes with heavy drinking.

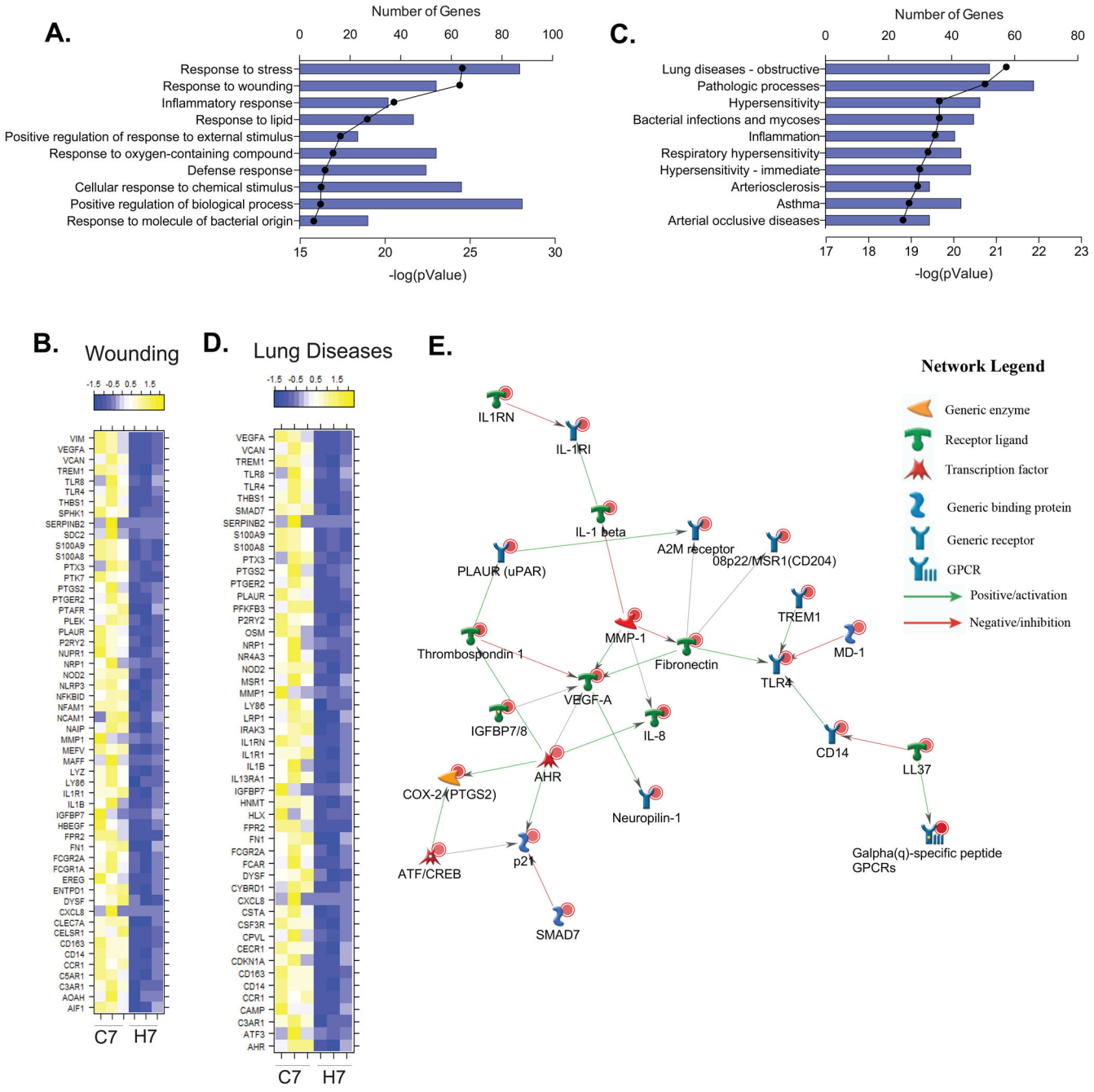

Figure 2. Chronic heavy alcohol consumption down-regulates genes that promote wound healing and contribute to obstructive lung diseases compared to controls and moderate drinkers

(A) Bar graph displaying the 10 most significant GO terms associated with the 170 genes downregulated with heavy ethanol consumption (H7) compared to controls and moderate drinkers (C7 and M7). Line represents the −log(p value) associated with each GO term. (B) Heatmap of DEGs between H7 and C7 in the “response to wounding” GO term. (C) The 10 most significant diseases by biomarkers associated with the 170 genes downregulated with heavy ethanol consumption (H7) compared to controls and moderate drinkers (C7 and M7). (D) Heatmap of DEGs between H7 and C7 that mapped to “lung diseases-obstructive”

category. (E) Network of DEGs that mapped to “obstructive lung diseases” and show direct interactions.

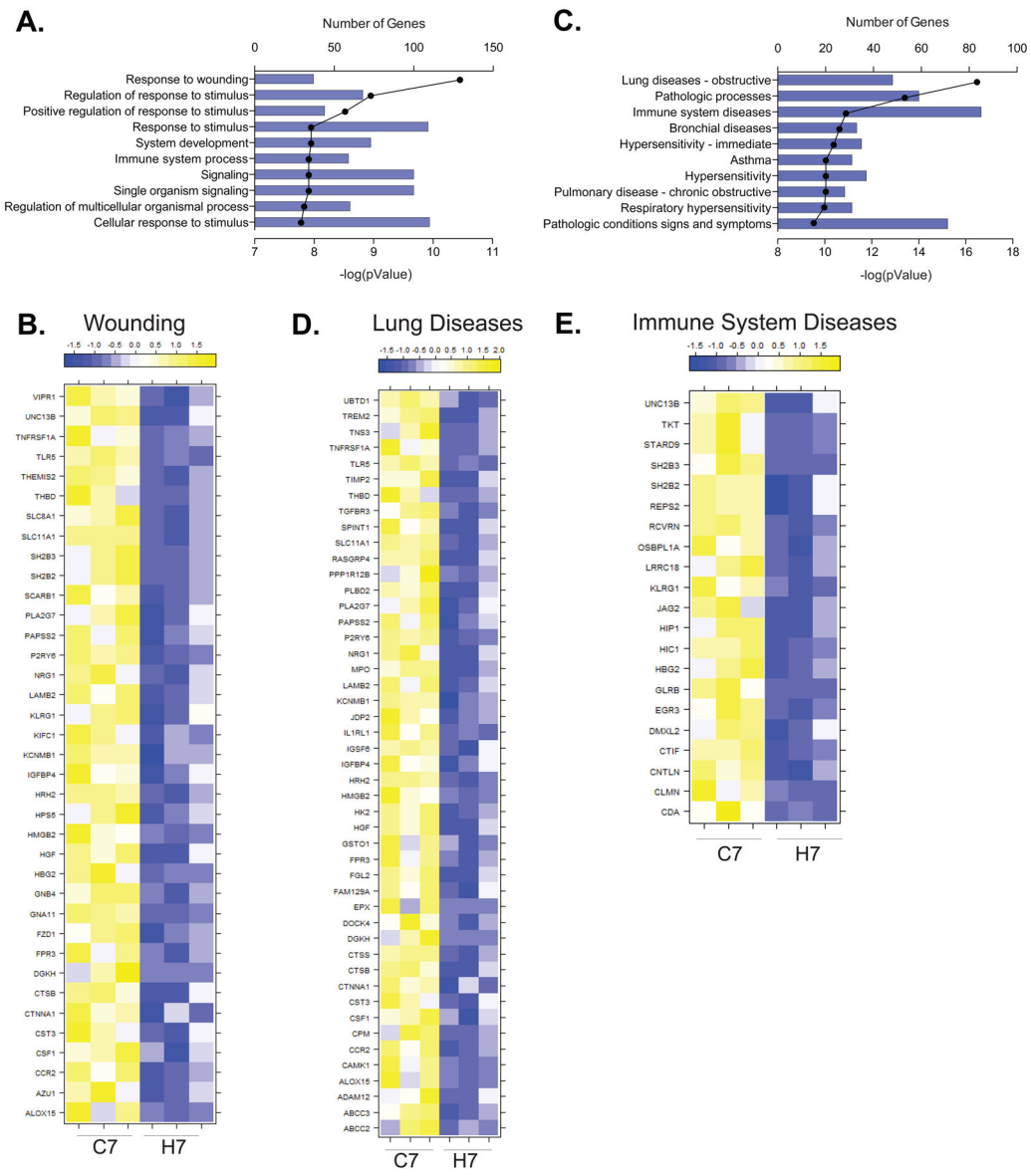

Figure 3. Alcohol abuse uniquely down-regulates additional genes that promote wound healing and contribute to obstructive lung diseases

(A) Bar graph displaying the 10 most significant GO terms to which the 186 DEGs downregulated with heavy ethanol consumption (H7) compared to controls only (C7). Line represents the −log(p value) for each GO term. (B) Heatmap of DEGs between H7 and C7 mapping to the GO term “response to wounding”. (C) Bar graph displaying the 10 most significant Diseases by Biomarker to which the 186 DEGs downregulated with heavy drinking (H7) compared to controls (C7) only. Line represents the −log (p value) for each disease category. (D) Heatmap of the DEGs between H7 and C7 mapping to the “lung diseases-obstructive” disease category. (E) Heat map of the 21 DEGs between H7 and C7 mapping to “immune system diseases”.

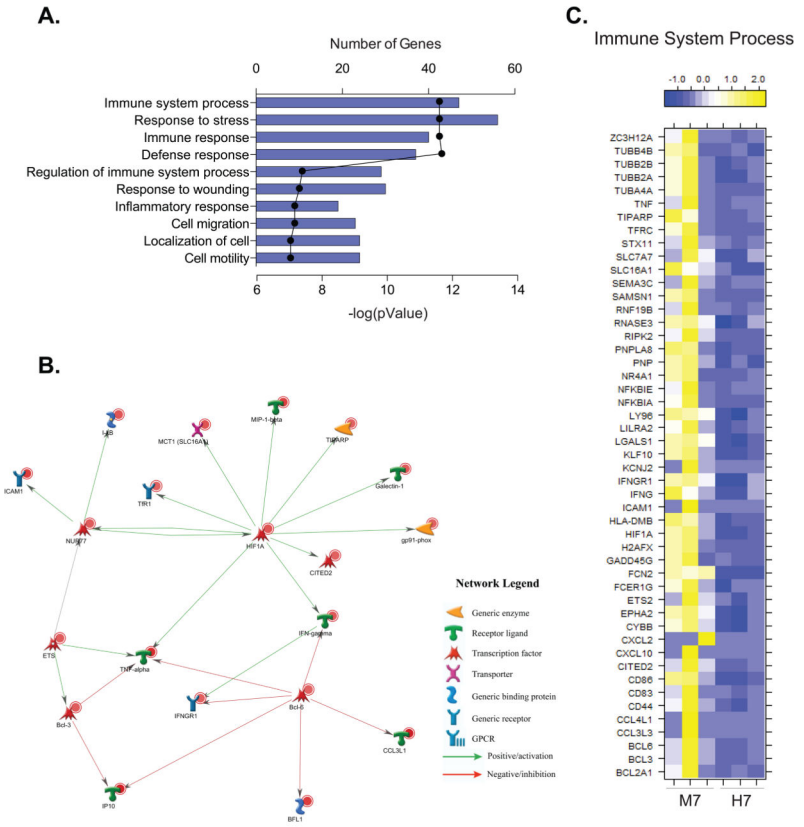

Figure 4. Heavy alcohol consumption downregulates genes associated with regulation of the immune system

(A) Bar graph displaying the top 10 significant GO terms associated with the 122 genes downregulated with heavy ethanol consumption (H7) compared to moderate drinkers (M7) only. Line represents the −log(p value) of each GO term. (B) Network image of DEGs that mapped to the GO term “immune system processes” and directly interact with one another. (C) Heatmap of the DEGs between H7 and M7 that mapped to the “immune system processes” GO term.

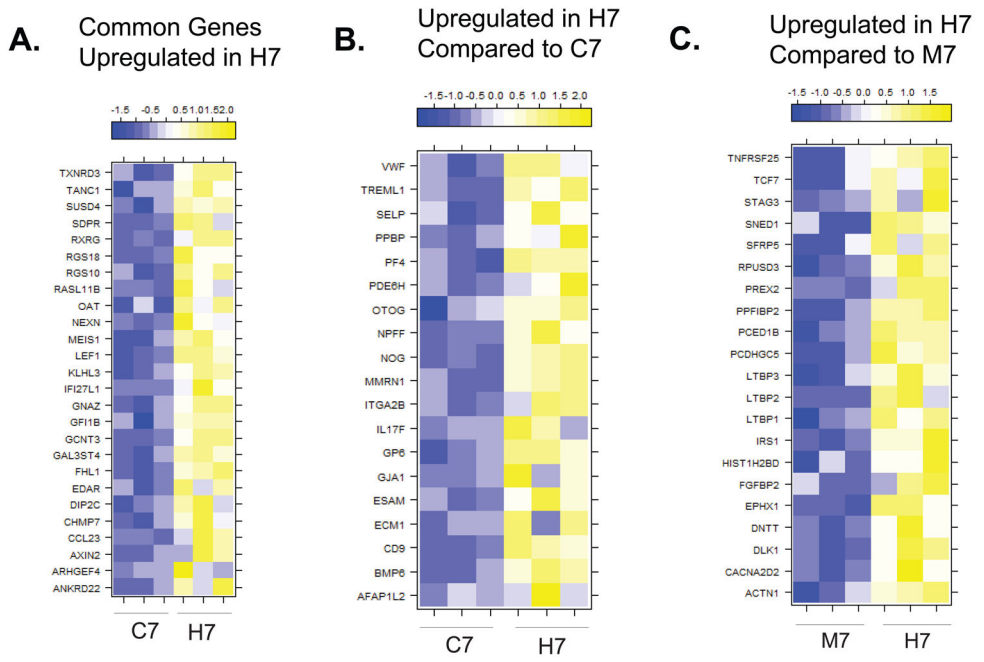

Figure 5. Alcohol abuse upregulates genes that interfere with wound healing and contribute to cancer

Heatmaps of the DEGs upregulated with heavy ethanol consumption (H7) compared to: (A) both controls (C7) and moderate (M7) drinkers; (B) controls (C7) only and mapped to the GO term “response to wounding”; and (C) moderate (M7) drinkers only and mapped to the GO term “neuro-ectodermal tumors”.

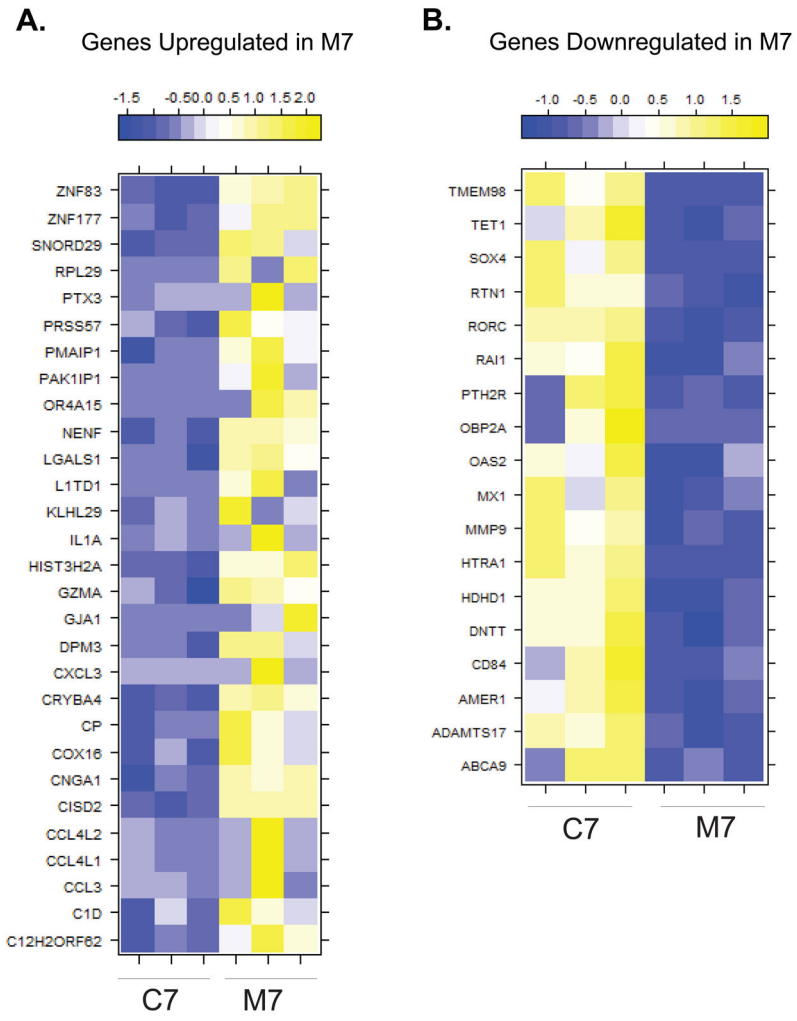

Figure 6. Moderate ethanol consumption modulates genes associated with immune response

(A) Heatmap of the DEGs uniquely activated with moderate drinking (M7) compared to controls (C7). (B) Heatmap of the DEGs uniquely repressed with moderate drinking (M7) compared to controls (C7).

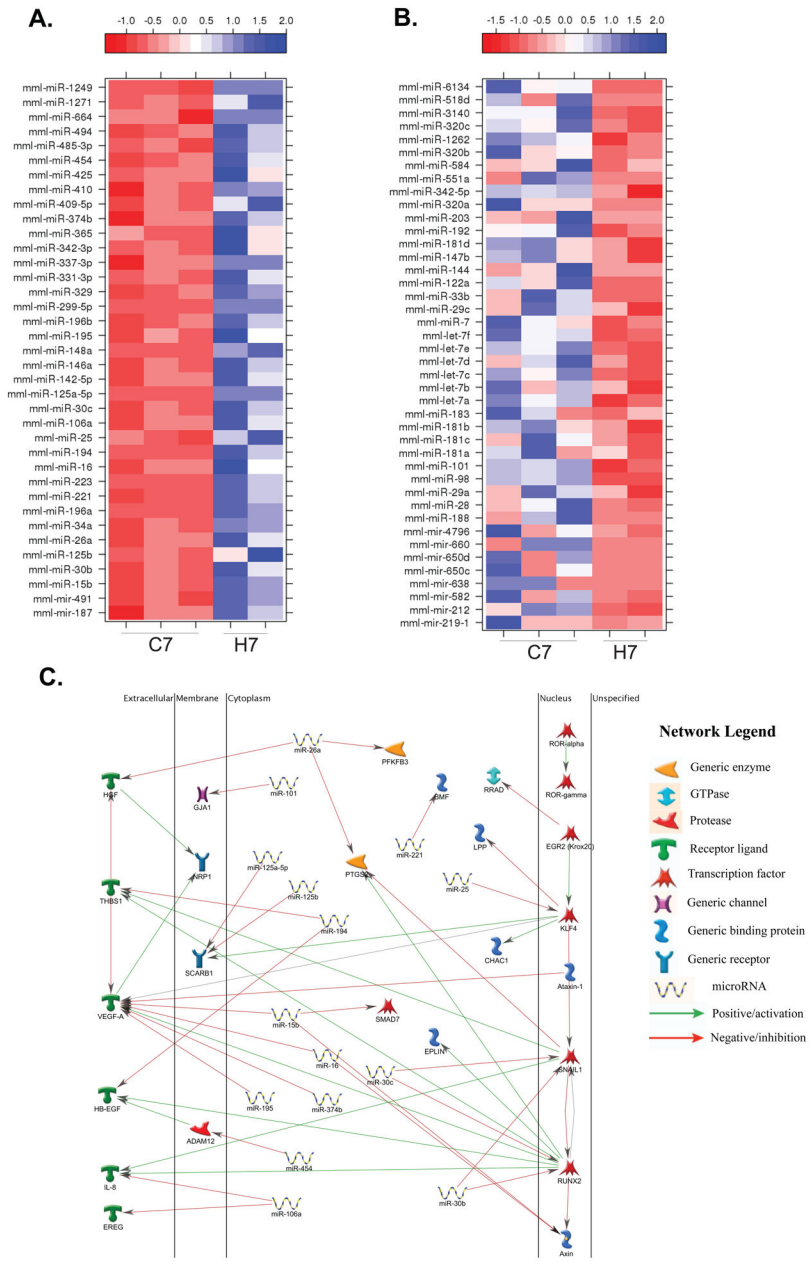

Figure 7. Heavy ethanol consumption changes expression of several microRNA

Heatmaps of the (A) upregulated and (B) downregulated microRNAs with heavy drinking (H7) compared to controls (C7). (C) Network image of a subset of the differentially expressed microRNAs and their mRNA targets that were both differentially expressed in our study.

Table 1

mRNA-miRNA pairs in our dataset[1]

Differentially Expressed MicroRNAs	Differentially Expressed mRNA Targets
Down-regulated	*Up-regulated*
miR-144	AXIN2
miR-203	AFAP1L2, ROBO2
miR-183	ROBO2
miR-29c	SH3PXD2A
miR-29a	SH3PXD2A
miR-101	GJA1
miR-29b	SH3PXD2A
Up-regulated	*Down-regulated*
miR-26a	HGF, PTGS2, NTN4, RP2, RAB3IP, CHAC1, PFKB3, DAPK1
miR-25	GRHL1, KLF4, FAM20C
miR-142-5p	GAS7, BVES, ATP13A3, PRRG4, RTN1, LPP, PRDM8, NRG1
miR-374b	GAS7, VEGFA, ATXN1, DUSP6, DUSP8
miR-125b	SCARB1, CCR2, FAM129B, C19orf39
miR-410	RORA, SMAD7, RAPGEF2, SASH1, SNAI1, PTX3
miR-485-3p	BAIAP2
miR-16	SMAD7, TNFSF13B, VEGFA, ATP13A3, RASGEF1B
miR-425	FSCN1
miR-30c	SH2B3, SNAI1, RUNX2, HLX, EAF1, RRAD
miR-106a	LIMA1, NTN4, OSM, WFS1, EGR2, EREG, DOCK4, FAM129A, RORC, IL8
miR-342-3p	ZAK, NEURL1B
miR-494	IRAK3, ZFHX3
miR-125a-5p	SCARB1, CCR2, FAM129B, C19orf38
miR-148a	NRP1, B4GALT5, SESTD1
miR-15b	SMAD7, TNFSF13B, VEGFA, ATP13A3, RASGEF1B, AATK
miR-221	BMF, NRG1
miR-365	KCNQ1
miR-454	ZAK, ATXN1, RTN1, ADAM12, ACSL1, WDFY3, MB21D2, TMEM170B
miR-195	SMAD7, TNFSF13B, VEGFA, ATP13A3, RASGEF1B, AATK
miR-329	ATXN1
miR-34a	CLEC10A, REPS2
miR-30b	SH2B3, SNAI1, RUNX2, HLX, EAF1, RRAD
miR-194	HBEGF, THBS1, ZFHX3
miR-223	OLFM1, SLC8A1

[1] Table shows the downregulated miRNAs that target upregulated mRNAs as well as the upregulated miRNAs that target downregulated mRNAs in our datasets.